TASTER RESPONSES TO ACIDITY IN WHITE WINES

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Dissertation to obtain the Master's Degree in

VITICULTURE AND ENOLOGY – DOUBLE DEGREE

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2017

ACKNOWLEDGMENTS

Here I want to express my most sincere thanks and gratitude to all the people that help me in this road till the end of it.
First of all the Professor Malfeito Ferreira that gave me another point of view on wines and life, how to deal with in a team and gave so many different new experiences.
The Professor Battistutta Franco for his help and assistance. To the Professor Mariana Mota for her fundamental presence and participation.
The Professor Jorge Ricardo da Silva, Catarino Sofia and Carlos Lopes for their disposability and all the pleasure talking.
To Mister Antonio, D. Lena and D. Manuela for their help and patience.
Many thanks to all the people that, with patience and constancy, come to the trials and have made a fundamental contribution to this work.

To all my closest friends in Lisbon that help me during an entire year and not only: Lorenzo, Simone, Elisabetta, Deniz, Janin, Lorenza, Tobias, Hugo and Maria.

And a special thanks goes to my family: Cristiana, Giovanni and Elisa. Without them all this couldn't been possible. They gave me the tools and the strength.
Thanks to my uncle Claudio that always spurred me. To Roberto for his complicity and encouragements. At the end but not of importance to my grandfather Giulio. He always gave me more than he expected and I absorb more than I could ever imagine.

I want to really thank you all. I'm happy that we crossed our lines.

ABSTRACT

The aim of this study was to evaluate the responses of a panel of tasters trained to acidity in white wines. The training of tastes and sensations of the mouth was performed in relation to the acidity, sweetness, bitterness and astringency. Next, the tasters were segmented according to vinotype, sensitivity to PROP (6-n-propylthiouracil) and saliva flow. This panel was used to determine the detection and recognition thresholds of tartaric, malic and lactic acids in white wine with 4.2 g / L of total acidity.

The detection and recognition thresholds were 1.05 g/L and 1.32 g/L for tartaric acid, 0.85 g/L and 1.06 g/L for malic acid and 1.12 g/L and 1.30 g/L for lactic acid, respectively. These acids were added to an Arinto wine at concentrations 1.5 times higher than the recognition threshold, obtaining responses regarding the flavor effect considering intensity, persistence, salinity and appreciation. There were no differences ($p<0.05$) in relation to the first 3 parameters, while the appreciation was higher in relation to lactic and malic acids. The statistical treatment of the responses according to the segmentation revealed relationships ($p<0.05$) between saliva flow and sensitivity to PROP, and between saliva flow and the tartaric acid recognition threshold. The acidity appreciation was higher in men than in women.

The results obtained can be used by the wine industry in the sense of adapting the white wines to the preference of the consumers, taking into account the recent trend towards the consumption of cold climate wines.

Keywords: Wine tasting, organic acids, Sourness, Sensory Preferences, Sensory Threshold, Taste Phenotype.

RESUMO

O objectivo deste estudo foi a avaliar as respostas de um painel de provadores treinado à acidez em vinhos brancos. O treino dos gostos e sensações de boca foi executado em relação à acidez, doçura, amargor e adstringência. Em seguida procedeu-se à segmentação dos provadores de acordo com o vinotype, sensibilidade ao PROP (6-n-propylthiouracil) e fluxo de saliva. Este painel foi usado para determinar os limiares de detecção e reconhecimento dos ácidos tartárico, málico e láctico em vinho branco com 4,2 g/L de acidez fixa.

Os limiares de detecção e reconhecimento obtidos foram de 1,05 g/L e 1,32 g/L para o ácido tartárico, 0,85 g/L e 1,06 g/L para o ácido málico e de 1,12 g/L e 1,30 g/L para o ácido láctico, respectivamente. Estes ácidos foram adicionados a um vinho de Arinto, em concentrações 1,5 vezes superiores ao limiar de reconhecimento, obtendo-se respostas em relação ao efeito no sabor considerando a intensidade, persistência, salinidade e apreciação. Não se encontraram diferenças ($p < 0,05$) em relação aos 3 primeiros parâmetros, enquanto a apreciação foi mais elevada em relação aos ácidos láctico e málico. O tratamento estatístico das respostas de acordo com a segmentação revelou relações ($p<0,05$) entre o fluxo de saliva e a sensibilidade ao PROP, e entre o fluxo de saliva e o limiar de reconhecimento do ácido tartárico. A apreciação da acidez foi mais elevada em homens do que em mulheres.

Os resultados obtidos podem ser utilizados pela indústria dos vinhos no sentindo de adaptar os vinhos brancos à preferência pelos consumidores, tendo em conta a recente tendência para o consumo de vinhos clima de frio.

Palavras-chave: Degustação de vinho, ácidos orgânicos, Sourness, Preferências Sensoriais, Limiar Sensorial, Fenotipo de Gosto.

Resumo alargado

O objectivo deste estudo foi avaliar a resposta de um painel de provadores treinado à acidez em vinhos brancos. Os provadores foram escolhidos entre os estudantes e funcionários do ISA, tendo-se seleccionado 26 indivíduos. O treino foi feito em relação aos gostos e às sensações de boca como acidez, doçura, amargor e adstringência. Os provadores foram caracterizados no que respeita ao sexo, vinotype, resposta ao PROP (6-n-propylthiouracil) e fluxo de saliva. O vinotype foi estabelecido através de resposta a um questionário online (www.myvinotype.com). A resposta ao PROP foi obtida após prova de um composto amargo (propiltiuracil). O fluxo de saliva foi determinado após prova de uma solução de ácido cítrico e expectoração durante um minuto.

O painel foi usado para determinar os limiares de detecção e de reconhecimento dos ácidos tartárico, málico e láctico adicionados a um vinho com 4,2 g/l de acidez fixa, seguindo um procedimento de teste triangular em copos transparente INAO. Os resultados obtidos foram de 1,05 g/L e 1,32 g/L para o ácido tartárico, 0,85 g/L e 1,06 g/L para o ácido málico, 1,12 g/L e 1,3 g/L para o ácido láctico, respectivamente para os limiares de detecção e reconhecimento. Estes ácidos foram adicionados em concentrações 1,5 vezes superiores ao limiar de reconhecimento a um vinho base de Arinto, tendo os provadores avaliado o gosto em relação à sua intensidade, persistência, salinidade e apreciação. A comparação entre os ácidos (ácido tartárico = 1,95 g/L; ácido málico = 1,5 g/L; ácido láctico = 1,95 g/L) mostrou não haver diferenças em relação à intensidade, persistência e salinidade. Em relação à apreciação, os ácidos láctico e málico foram os mais apreciados.

A comparação entre as acidezes de diferentes vinhos foi feita usando 2 vinhos de regiões de clima muito diferente. Um provinha do Alentejo (região quente) e outro da Alemanha (região fria) da casta Riesling. Um terceiro vinho foi obtido pela adição de 1,5 g/l de cada um dos ácidos málico e láctico ao vinho alentejano de forma a compará-lo com o vinho da região fria. Os resultados foram obtidos pela medição da intensidade numa escala de estimativa de magnitudes.

Por fim, tentou-se perceber se havia relações entre os diferentes segmentos dos provadores e as respostas à acidez. Através do tratamento estatístico por ANOVA foi possível encontrar relações entre o fluxo de saliva e a sensibilidade ao PROP e o fluxo de saliva e o limiar de reconhecimento do ácido tartárico. Em conjunto, os homens mostraram uma maior apreciação pela acidez do que as mulheres.

Os resultados obtidos mostraram que não foi possível encontrar relações claras entre a acidez, considerada como factor isolado, e a apreciação dos vinhos. O vinho é uma matriz complexa no qual a interacção entre todos os componentes cria uma gama alargada de possíveis combinações. Esta diversidade justifica a continuação dos estudos tentando esclarecer o que determina a apreciação de vinhos com diferentes níveis de acidez fixa.

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LIST OF ABBREVIATION

pK_a – Logarithmic acid dissociation constant

k_d - Dissociation constant

TA – Total acidity

VA – Volatile acidity

[AH] – Un-dissociated acid concentration

MLF – Malolactic fermentation

MW – Molecular weight

meq/L – Milliequivalents per liter

HPLC – High performance liquid chromatography

PROP – 6-n-propylthiouracil

HF – High-flow rate

LF – Low-flow rate

ME – Magnitude estimation

Tart. – Tartaric acid

Malic. – Malic acid

Lact. – Lactic acid

Appr. – Appreciation

Pers. – Persistence

Sali. – Salinity

Inten. – Intensity

C.P. – Castelo de Pias

Ries. – Riesling

SD – Standard Deviation

SE – Standard Error

1. INTRODUCTION

The first evidence of wine production has been found between 6000 and 8000 B.C. in Georgia, Iran, Greece and Armenia. At the beginning it was considered and used as spontaneous fruit and just after the transition from nomadism to sedentarism, it began a cultivated plant (Trevisan, 2011). The wine as we know it nowadays, its spread and develop, begun with Egiptians and then with the Greeck and Romans. With them, the Catholicism it has become the most important religion in Europe and in half of the known world, and it helped the vines to be saved and disseminated in large areas. By that time, the wine assumed a several roles in society and acquired importance in the daily life as central figure in religious practices, potential medicinal properties (Trevisan, 2011) and its exhilarating effect.

1.1. THE CONCEPT OF WINE QUALITY AND APPRECIATION

Within the field of food science, the concept of perceived quality has attracted interest for decades (Saenz-Navajas *et al*., 2012). The overall aim of many grape and wine research studies is to improve wine 'quality': providing ways of understanding, altering and controlling compounds that affect wine sensory properties through viticulture and winemaking to make consistently better wines. To produce wines free of deficiencies and with sensory characteristics that appeal to consumers is of fundamental importance. The application of rigorous sensory evaluation to assist in this goal has become of increasing significance, especially in a global marketplace (Francis and Williamson, 2015).

The concept of quality can be difficult to define and in literature can be found so many different definitions. In particular, wine, if compared with other beverages, has a wide range of aromas determined by a several variables such as grape varieties, raw material, winemaking methods, viticultural practices, geographical origins or vintage (Maitre *et al.*, 2010). Quality is hard to define because of the lack of general agreement. In fact persons differ in the wine quality perception because its holistic feature that has roots in the results of individually conceptions and previous experiences, and include all different levels of quality in one judgment (Hopfer and Heymann, 2014).

The quality of the wine comprises a number of dimensions, both intrinsic to wine tasting and extrinsic to it. For that, quality is the result of overall perception of the wine properties (Pilar *et al.,* 2012). The extrinsic factors include the grape growing, the winemaking and the basic definition of the wine quality as the lack of technical mistakes and its drinkability. The intrinsic factors are indeed defined as drinking experiences which in turn include factors as pleasure, aroma, mouthfeel, appearance or factors either important for the involvement of interest as

origin, variety, typicality and potential (Hopfer and Heymann, 2014). Both the factors influence each other and, at the end, they produce a common judgment. Intervening on one of them is possible to modify the final result. This is true even if they don't have the same weight where the intrinsic tasting experience has a quite more influence on the general assessment.

What the consumers are looking for is "enjoy" the wine and especially, parallel with the improving of the drinking experience, move their consumption towards to quality wines. Mainly the consumers with a low degree of wine knowledge, rely and trust the experts of the sector. The experts are known acting more analytically and based, hopefully, on previous studies and experienced. So the consumers trust the professionals and they look at them as guidance. It's here that the importance of the wine competitions entry in the equation. The awards offer to the markets the possibility of having sure choices in findings "enjoyable" wines. Such as all the products, the wine is subjected to high variability in liking and perceived quality, even between experts. Therefore in competitions the awards are a matter of preferences (Hopfer and Heymann, 2014).

1.1.1. The Influence of Wine Competitions on Wine Appreciation

In the world scene of today, where the offer of wines on the market is huge, the purpose of the wine competitions is to give parameters and advices in the open range of possibilities that consumers can face every day and for every occasion. They can be either be useful to guide the consumer and even to move the market in certain directions. On the other hand, these wines competitions tend to standardize a style or a tendency with the consequence to reward wines and exclude others not only on the base of quality, but mainly on a specific footprint. The tendency of the last years of wine competition, is to attribute the medals to balanced favor profile with marginal notes of vegetal-green, chemical, earthy or sulfur characters, aromas of fruit and oak, hot/full mouthfeel (generally related to the alcohol content), low bitterness and high sweetness (Hopfer and Heymann., 2014). During the last years, the awards had moved the markets towards wines with the features mentioned above. By using the enological techniques and enological products, it's easier for the wineries to shape the wine as the trend is pushing. But this "style" is now producing a standardization, favoring "easy" international commercial wines in opposition to the "difficult" European classic wines (Loureiro *et al.*, 2016).

Generally, the wine competitions publish in their websites only the results and the awards without indicating the grades gave to every single parameter. The exception is the Mundus Vini that publishes them in their site (www.meininger.de/en/mundus-vini). Here we report an example of the average distribution of the main features of the wines awarded Gran Gold and Gold of the year 2015 (Figure 1.1). The main considered features in this competition

concern an overall evaluation (Harmonius, Complex, Potential, Body), a mouthfeel evaluation (Acidity, Sweetness, Bitterness, Astringency) and an olfactory test (Cherry, Jammy, Dried Fruit, Smoky, Oak; Barnyard, Berries, etc.)

Giving more importance to flavors and sweetness, the acidity is not well considered, even if is one of the most important components of the wine. The altering of the palatability, can be now achieved through the addiction of a wide range of enological products. This trend is conflicting with the tendency to use always more healthy and biological food. In the closest future, we may foresee that wine manipulation will be even more important because new markets are opening.

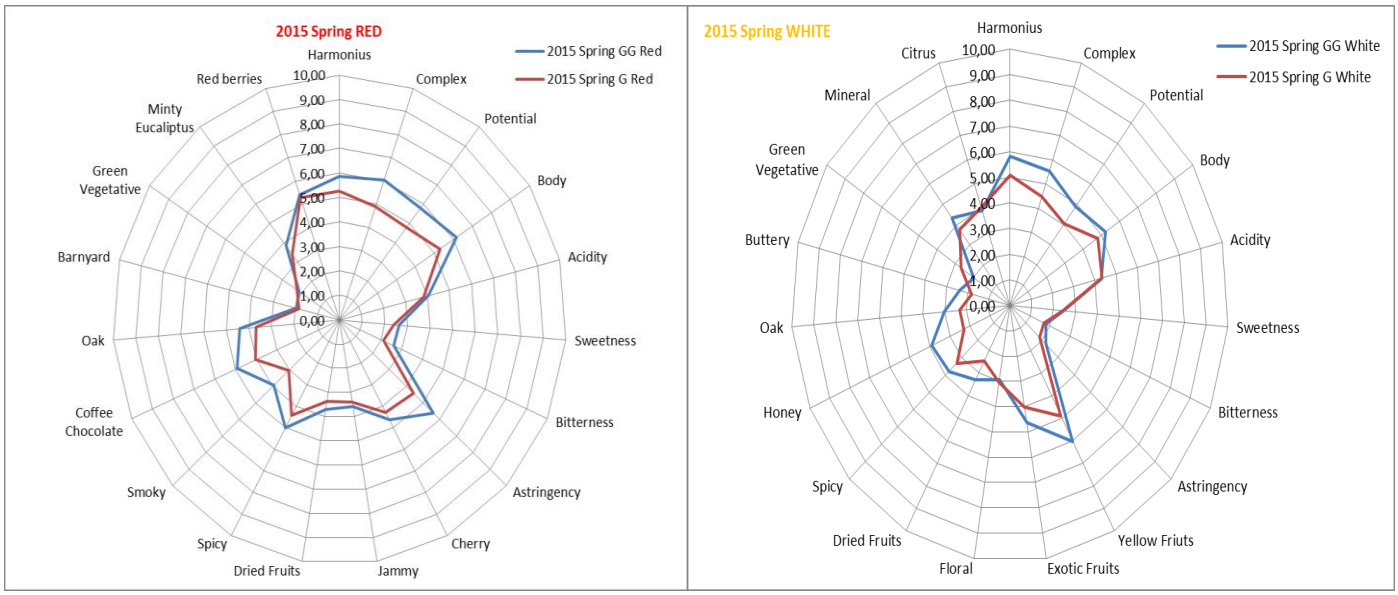


Figure 1.1. Sensory profiles of Wines awarded of Gold and Great Gold medals in Mundus Vini challenge (Spring tasting 2015, red and white wines).

1.1.2. The Influence of Consumer Segmentation on Wine Appreciation

The wine can give sensations that are correlated with the primordial reactions and some compounds, instead of others, communicate different messages to our brain. In fact the sense of taste controls one of the most important decisions animals make: whether to eat or reject a foreign substance (Mennella *et al*., 2011). Already from the birth, the humans try naturally to find sweets foods because linked to energy. At the same time, salty food correspond to minerals, savory to proteins and bitter and sour respectively to toxins and unripe fruits. The sweet elements in a wine are the result of residual sugars or alcohol and polysaccharides in dry wines. Acidity instead is the result, mainly, of the two dominate grape acids, tartaric and malic. Both astringency and bitterness are the result of phenolic compounds derived from the grape, oak barrels or both.

On our tongues there exist a number of taste buds, each containing a variety of taste receptors. These detect five different modalities, although there is some discussion about whether there might be more. They are sweet, sour, bitter, salty and umami (the sourness of amino acids). As well as these, there are receptors for heat and touch. In wine the taste and tactile components are perceived according to the capacity of our tongue and mouth to feel the different sensations and the contact with substances (Chandrashekar *et al*., 2006). These sensations are felt differently by persons and recent developments sensory science relies on the evaluations of responses according to consumer segmentation. Classical taster segments include gender, age or knowledge but other factors are now under focus as described below.

Taster phenotype

The sensitivity to the bitter taste of 6-n-propylthiouracil (PROP) is a heritable trait (Drewnowski, 1997). The subjects can be divided in three groups depending on the PROP detection threshold and the mean ratio of intensity. The classification is divided in 3 groups: super-tasters, tasters, non-tasters. Participants were classified as non-tasters, tasters or super-tasters based on the bitterness rating they assigned to the 0.32 mM PROP solution using the magnitude estimation (ME) line (non-taster: ≤15.5; taster: >15.5 and <51; super-taster: ≥ 51) (Pickering *et al*., 2004). The distinction is made on recent anatomical studies that takes in account the amount of fungiform papillae, taste buds, their number and density. The sensitivity to PROP is associated with increased acuity for other bitter compounds, and for sweet taste. The response to the PROP may predict the hedonic pleasure to sweet taste. It has been shown that PROP non-tasters were always sweet likers, and sweet dislikers were almost always tasters or super-tasters (Bartoshuk *et al.,* 1994).

Vinotype

The previous studies on taste phenotype were the base for the development of the so-called Vinotype which is an online test based on a series of questions that helps to determine the sensory sensitivities and tolerances to wine (www.myvinotype.com). The result is a combination of personal preferences about wine. It gives an understanding of your own sensory sensitivities. This test is has the purpose of helping the subject in the choice of the right wine in stores or in restaurants. Four different vinotypes types can be attributed:

i) SWEET – At the top of the scale in terms of sensitivity and usually very picky about wines. Trend to prefer sweet wines and in general to sweet foods or drinks.

ii) HYPERSENSITIVE – Subjects are very sensitive. Range that contain the ones more conservatives, affectionate to the well-known wines, and the more adventurous that love to discover and try new wines but with clear parameters.

iii) SENSITIVE – The classification that include the major part of the subjects with a medium sensitivity spectrum. That also means, able to enjoy a larger segment of wine styles. People flexible, adaptable, adventurous and able to find the right wine.
iv) TOLERANT – The subject crave for intensity and lots of flavor and can't quite understand how other people drink weak wines.

Saliva production
Saliva can affect perception of taste trough titration, dilution and precipitation of stimuli. The stimulation by oral manipulation or ingestion of stimuli causes the salivary low rate to increase (Fischer *et al*., 1994). Saliva is the first physiological secretion induced by ingestion of foods or beverages. Its reaction play an extensive role in the oral cavity and in taste perception. Every individual react differently to the stimuli and as well the production of the saliva vary in flow and degree of response to oral stimuli (Fischer *et al.,* 1994). Saliva is the responsible for supply the background environment response to perception and assess of taste stimuli. Depending on the rate, can be defined two types of subjects: with high-flow rate (HF) and low-flow rate (LF). This is a major differentiation which however can't explain all the relative responses to the different stimuli. Acids seems sourer to the subjects that have lower saliva flow rate and with lower salivary pH (Fischer *et al*., 1994).
The Saliva test, by using the SPI (Saliva Precipitation Index), measure the reactivity of salivary proteins towards wine polyphenols (Rinaldi *et al*., 2012). This interaction causes complex formation and their precipitation with consequently reduction of the lubricating properties. This lead to sensations of dryness, hardness and constriction in the mouth.
The saliva production can be estimated by the weight of saliva elicited in response to 10 ml of 4 g/l of citric solution expectorated after 10s. Saliva is then collected, spitting for one minute in a weighed container (Ishikawa and Noble, 1995). Their results showed that in astringency perception, there are differences between the subjects depending on the salivary flow. In our case the interest falls to the white wines where people with low parotid flow rates, perceive astringency with a higher maximum intensity, longer latency and total duration (Smith *et al*., 1996).

1.1.3. Sensation Measurement
Stevens (Figure 1.2), in the 1950's, described the technique to measure individual's judgement of stimuli that vary widely in intensity (Schifferstein, 2012). The *magnitude estimation* is a psychophysical scaling International Standard technique where tasters can assign numerical values to the estimated intensity of a feature and the evaluation of sensory attributes (ISO 11056:1999). The only must that has to be follow by the assessors is that the value assigned should be conform to a ratio principle.

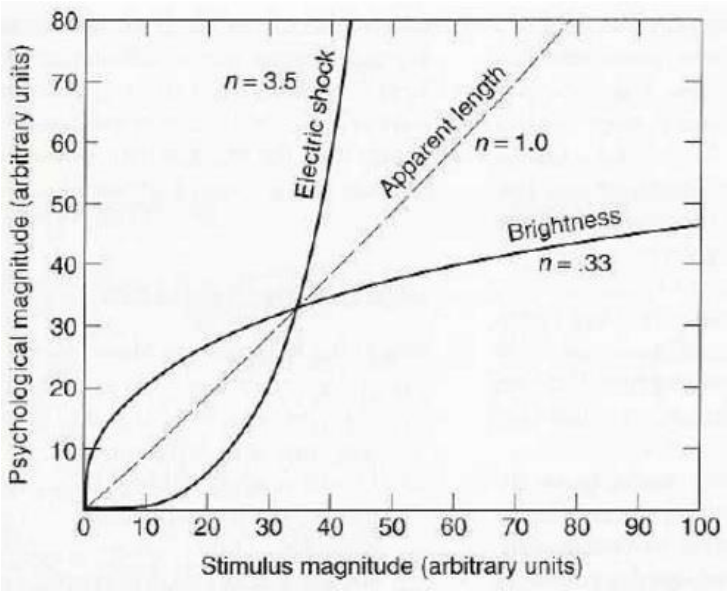


Figure 1.2. Steven's Power Law (Goldstein, 2009).

This scale can be used for attributes such as intensity, pleasantness or acceptability. The magnitude estimation is useful when the time available and number of assessors are limited. It is a flexible scale and the tasters can apply it to a wide range of samples and attributes. It allows the assessors to have an infinite number of categories and avoid the "end-effect". This can happen when the assessors are obliged to classify samples perceived as being different into the same category (https://www.iso.org/obp/ui/#iso:std:iso:11056:ed-1:v1:en). The length of the line it was of 102 mm. The subjects are free to choose, throughout the length of the line, the distance from the left border that more suits or represent the intensity of the sensation.

The magnitude estimation it has also some side effects. It is not the most efficient for determining small differences between stimuli or for conducting assessments in the vicinity of a detection threshold. This methodology obtains magnitude estimations and their statistical interpretation, being widely applied in food and wine studies (Green *et al*., 1996).

1.2. WINE ACIDITY

Wines are composed by 80%–90% water, 0.1%–20% sugar, with pH determined by a balance between 0.3%–1% acids (tartaric, malic, citric, lactic) and mildly alkaline alcohol (8%–20% ethanol, glycerol), organic compounds (0.3%–1% flavor compounds, such as anthocyanins, tannins, and flavonoids), and mineral cations (0.1%–0.3% potassium, sodium, calcium, and magnesium) (Jackson, 1994). All these main components contribute to create the broad flavors and mouthfeel feelings. In this section we will focus on the description of wine acidity and its influence on taste given that it is the aim of our studies.

1.2.1. Evolution of Organic Acids in the Grapes

The acidity in the grapes, it is the result of all of the complex physiological and biochemical phenomena that happens during the maturity of the raw material related to the environmental conditions. The concept of the acidity in wine has to be clarified by separating it and explaining the two different kinds. The acidity attributed to the organic acids, perception positively correlated to the perceived sourness, and the one of the pH, instead negatively correlated. The acidity of the organic acids, it is subject to an evolution during the growing season (Figure 1.3).

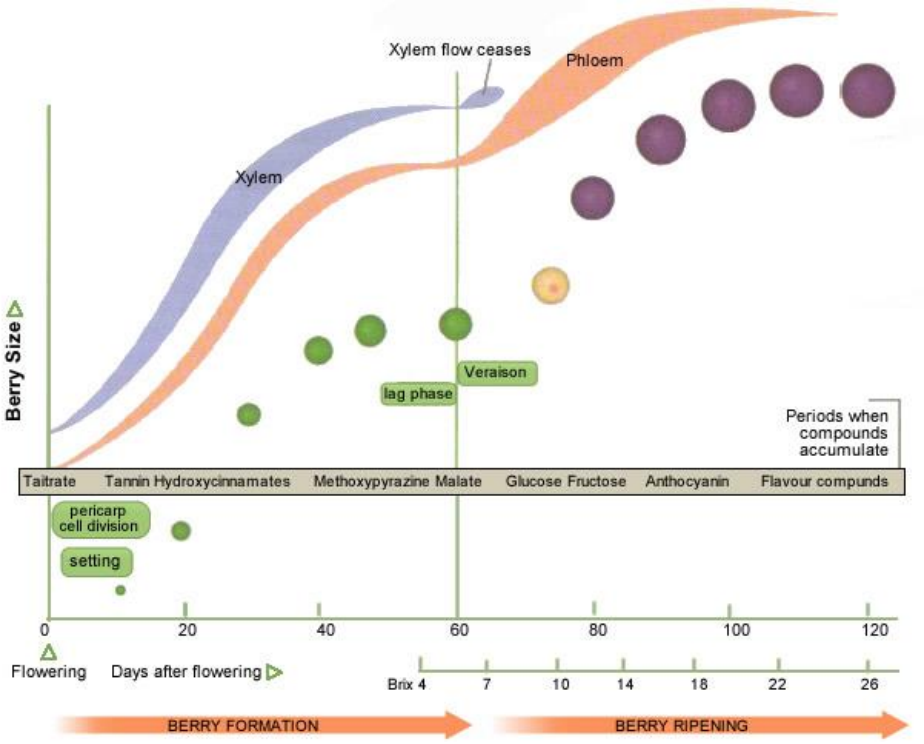


Figure 1.3. Evolution of the Grape Development (Keller, 2010).

During the berry development, three stages occur. In stage I, starting with the fruit set, happens the enhancement of the grapes caused by the cellular division. In the stage II, called the Lag Phase, there is a stop in the berry growing because the cells division stops as well and it begins the enlargements of the same ones. With the Stage III starts the *veraison* where the berries metabolize the acids, change color and accumulate sugar. The presence of the acids in the grapes, is a way to defend the seeds from the consumption of vertebrate animals or birds. As well, the tannins have the same function. In the Lag Phase the amount of the acids become to decrease, the sugar content increase and so the attractiveness of the grapes it starts to increase (Keller, 2010). In the maturation phase the most important acid is the malic where it fill a dominant role in the ripening even if the tartaric acid is the most present one.

Despite they're both being synthesized during the first phase, they follow a different patter during ripening. In fact the tartaric content of grapes varies very little when instead the malic acid follow the decrease in total acidity. The rate between this two acids at the end of the ripening, is important because it influence the final pH of the wine and the final titratable acidity. But, where the tartaric acid is more stable and do not follow significant modifications, the malic is very sensible to the vintage's conditions (Jackson, 1994).

Influence of the climate on wine acidity

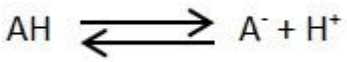
The changing of the climate is having a heavy impact on the ecosystem directly through shifting climatic controls and indirect on lands used for the viticulture (Hannah, 2013). Viticulture is a good test case to measuring this impact as is sensitive to climate and concentrate in a defined range (Hannah, 2013). The climate has enormous effect on vine phenology, grapes and wines composition, the vinification, the chemistry and the microbiology conditions of the wines. The rising temperature have already had a significant effect on the grape and wine industry (Mira-de-Orduña, 2010). The temperature especially has a considerable effect on the total acidity. This because while the main grape's acid, tartaric acid, is stable to the temperature, the malic acid is instead very sensible and, depending on temperature and maturity level, it can be strongly influenced. Indirectly the temperature affect the potassium accumulation. It has been suggested that potassium enters berry cells in exchange for protons and affect the pH of the grapes (Mira-De-Orduña, 2010). Over the last few decades, global climate change and variations to viticulture and oenology practices have determined a trend towards an increase in alcohol content and a reduction in total acidity of wines (Gobbi *et al*., 2012). As global climate change and variations in viticulture and oenology practices have resulted in a trend towards the reduction of the total acidity of wines (Gobbi *et al*., 2012). The possibility of biological acidification and ethanol education might have an important role in satisfying the growing consumer demand in the wine market (Fleet, 2008).

We already mentioned that a really hot season improve the possibility of an higher pH and lower total acidity. It leads to a lowering of the quality of varietal aromas in favor of the ones of post-fermentation. But the different acids seems to have different taste and persistence. It generally confirmed that in cold climates the pH have lower levels and the TA have higher levels than wines from warm regions.

1.2.2. Chemistry of Organic Acids

The organic acids are one of the most important components of the entire character and taste of wine (Zeravik *et al*., 2015) and "make major contributions to the composition, stability and organoleptic qualities of wines, especially white wines" (Ribereau-Gayon, 2006).

The property shared by all acids is the dissociation of protons when dissolved in water and it's called ionic dissociation (Neta, 2007). Can be represented as:



Where $[A^-]$ and $[H^+]$ are the equilibrium concentrations (molar) in the solution of the anionic form of the acid and its proton respectively. Can be then defined the K_d or dissociation-constant: $K_d=[A^-]\,[H^+] / [AH]$.

The acidity depends on the dissociation constant, or pK_a, that is the logarithmic relationship between the K_d and the pH (Margalit, 2012) and it permits to calculate the dissociated to un-dissociated acid ratio at a given pH. This value indicates the quantity of protons that the acid release to the solution. Lower it is, more protons are ceded to the solution and stronger is the acid. For the organic acid we considered tartaric has a pK_a around 3, which means it is pretty strong. Malic is around 3.4 and lactic is 3.8 so they can be considered weak acids (Table 1.1).

Table 1.1. Dissociation constants (pKa) and hydrophobicities (log P) for organic acids (adapted from Neta, 2007).

Acid	pK_{a1}	pK_{a2}	pK_{a3}	log P
Acetic	4.75			−0.17
Lactic	3.86			−0.62
Adipic	4.43	5.41		0.08
Fumaric	3.03	4.44		0.27
Malic	3.40	5.11		−1.26
Tartaric	2.98	4.34		−1.84
Succinic	4.19	5.50		−0.59
Citric	3.14	4.77	6.39	−1.72

The acids, and as well the bases, can be distinguish in hard and soft. They both dissociate in water but the strong ones are being completely dissociated meaning that they give all the protons to the solution. The hydrogen ions (H+) are formed when a dissolved acid partially separates (dissociates) in to hydrogen ions and related anions (A-). The strong ones do it more than soft ones, which are instead ionized in minimal part, and some protons remain in un-dissociated form. The acids are able to release one or more protons where the one or more dissociation-constants take place in different place.

That means that different acids at the same concentration, contribute with difference intensity to release protons to solution. Hence lower is the pK_a, greater is the acidity contribution to the pH (Margalit, 2014).

The Multiplying Factor (Table 1.2) is a formula based on tartaric acid and is the equivalent weight of the other acids divided by that of tartaric acid (EW = 75). Is used to convert the acid concentration (in g/L) into his equivalent weight as tartaric acid, multiplying its concentration for the appropriate converting factor (Margalit, 2014).

Table 1.2. Molecular weight (MW), Protons per Molecule, Equivalent Weight and Multiplying Factor for the main acids in wine. Adapted from Margalit (2012).

ACID	MW	Protons per Molecule	Equivalent Weight	Multiplying Factor
Tartaric	150	2	75	1.00
Malic	134	2	67	0.89
Lactic	90	1	90	1.20
Succinic	118	2	59	0.79
Fumaric	116	2	58	0.77
Citric	192	3	64	0.85
Acetic	60	1	60	0.80

The acids can be either preexisting from the grapes or as byproducts of the fermentation (Figure 1.4). The ones already present in the grapes are “natural” and have the freshest, purest acid tastes.

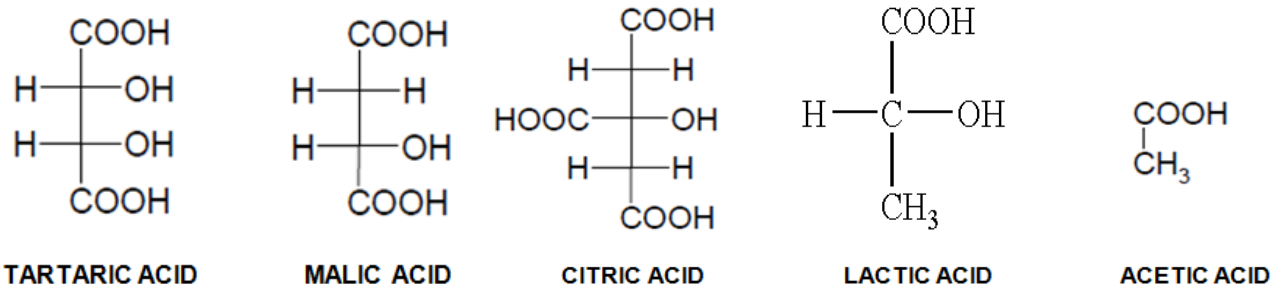


Figure 1.4. Structural formula of the main acids in the wines. Images from: Lianyungang Sunchem Co. Ltd.

Tartaric Acid

Is the most representative one in musts and wines and in the must, it has a concentration usually 3 times higher than wine. At the end of the vegetative growth phase it has a value around 15 g/L. In nature is not so widespread in every fruit except for grapes where is the predominant one. The one contained in the grapes it has form L (+) (Usseglio-Tomasset, 1995). Compared with the other acids is the most voluminous acid, (MW: 150.08684 g/mol) and strongly control the pH of wines.

During the fermentation precipitates as potassium bitartrate. This is happening by the increasing of alcohol level that insolubilize the acid. Depending on the area (north / south), the year (hot / cold), and the type of soil, generally it varies between 3 and 6 g/L; lower in the south and higher in the north.

It is an acid with 2 possibility of dissociation (diacid): pK_1: 3.04; pK_2 :4.37. Is the strongest one in the grapes and is the most important one for the problems that can cause to the wine due to the insolubility of the low solubility of its salts (Usseglio-Tomasset, 1995).

Malic Acid

Is already present in the grapes L (-) and more widespread in nature than tartaric, both in fruits and vegetables. The malic decrease during the ripening of the fruits, but with different speed according to the climate; faster in warmer and slower in cold ones. It's considered having a "green taste" and sharp. During the fermentation there is the opportunity to decrease it, by using the malolactic fermentation (MLF) where the malic acid is converted to the smoother lactic acid; the reduction proportion can reach a factor one to five. MW: 134.0874 g/mol.

Even malic acid is a diacid with two pK_a: pK_1 : 3.46 pK_2 : 5.13.

Citric Acid

It is naturally present in the grapes, even if not in higher quantity such as other fruits. It gives a taste of freshness and it helps to prevent ferric hazes. During the fermentation and MLF, the amount decrease reducing considerably. MW: 192.124 g/mol.

The acids that can be produced by the fermentation have milder, more complex tastes. The major fermentation acids are lactic, succinic and acetic.

Lactic acid

Already present in the grapes but in minimum quantity. Mainly found in the wines, as product of the lactic bacteria. It has two different property: it can reduce the harshness of the malic acid, but it can make easier the infection by certain lactic bacteria. The result could be smells of milk or butter. That's why some winemakers try to dissuade from doing the MLF and others try give a particular notes to their wines. MW: 90.08 g/mol.

Acetic Acid

The most important volatile acid present in the wine and is a byproduct of the alcoholic fermentation, the malolactic fermentation, the acetic and lactic bacteria. Its odor is of vinegar and a natural component of the wines even if small quantities but could be as well produced by certain bacteria. That's the main difference with the tartaric and the malic because those, being not volatile, can't evaporate and, by consequence, they are not able to interact with the receptors in the nose. Even the detection of the Volatile Acidity (VA), needs a different

process to be quantified. A small amount of acetic acid is considered normal as byproduct of microbial metabolism. MW: 60.05 g/mol.

Succinic Acid

It is formed during the fermentation by yeast and it is present only in trace. It contribute to total acidity and its taste is a mixture of acid, salty and bitterness. MW: 118.09 g/mol.

Citramalic, dimethylglyceric, galacturonic, glucuronic, gluconic, ketoglutaric, mucic, oxalic, and pyruvic acids are also found in grape and many other wines in trace amounts and contribute to total acidity. In particular, *gluconic acid* can be used as indicator of *Botrytis Cinerea* and it has an important role in the organoleptic properties of wines. It either can be considered a decrease in the quality of grapes (Grey Rot), or high quality in dessert wines (Zeravik *et al*., 2015).

1.2.3. pH

The pH instead, is a measurement of the number of ions H+ (protons) and “expresses the acid strength of the wine” (Puckette and Hammack, 2015) and it goes usually from 2.8 and 4.0 (Figure 1.5). Is defined as the negative logarithm of the hydrogen ion concentration in gram-atoms per liter.

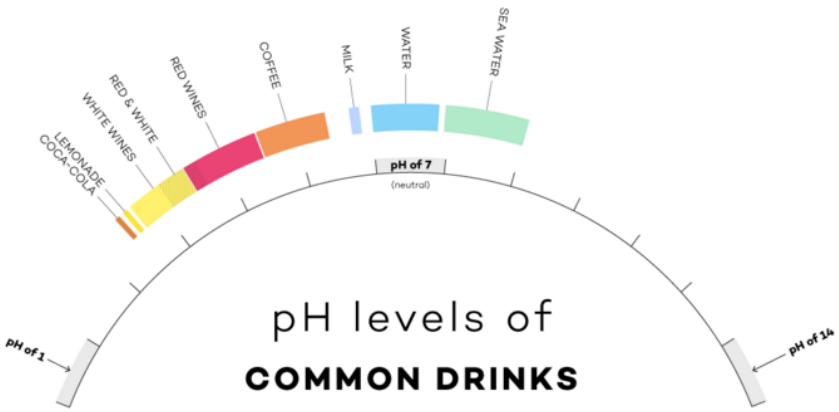


Figure 1.5. pH levels of common drinks (image from: Wine Folly: The Essential Guide to Wine).

Being an expression of logarithmic scale lower is the value, higher is the intensity. As logarithmic scale, a change in one point, correspond to a solution 10 times more or less intense. The scale is from 0 to 14, where all the values under 7 are acids and higher are basics. This parameter can change depending on the maturation rate of the grapes, on the environmental conditions and soil type.

The pH reflect the actual proton concentration in solution, which is not necessarily the total acid concentration. Both the musts and the wines are known as acidbasics buffer solution that are able to restore possible modification of pH. Usually the pH for white wines are included between 3 and 3.3. While instead for the reds the average is little bit higher, 3.3 to 3.6 (Figure 1.6). A low level of pH is generally more indicated for any kind of wine because it helps to reduce the risks of oxidation and microbial spoilage. Even the TA is important in this role inasmuch it able to change the pH.

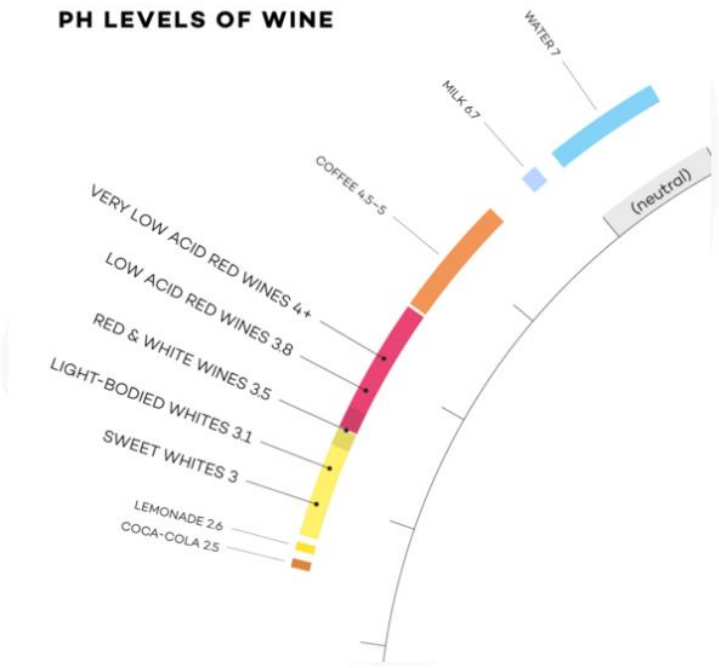


Figure 1.6. pH levels of wine (image from: Wine Folly: The Essential Guide to Wine).

1.2.4. Methods to Determine the Acidity

The acidity can be measured by different methods with different purpose: total acidity TA and potential of hydrogen (pH). The total acidity of the wine is the sum of its titratable acidities when it is titrated to pH 7 against a standard alkaline solution. Carbon dioxide is not included in the total acidity. With titratable acidity is defined the capability of the acids in the wines to neutralize an alkaline substance. But usually the amount of acidity is expressed in titratable acidity that consider the sum of all the organic acids. Even if TA and pH are not the same thing, they are related. A solution containing a relative higher quantity of weaker acids, such as malic, will generally have a lower pH. The methods to detect both the parameters are defined by the “Compendium of International Methods of Analysis – OIV” COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS – OIV (www.oiv.int/public/medias/4231/compendium-2016-en-vol1.pdf).

Total Acidity

Definition: The total acidity of the wine is the sum of its titratable acidities when it is titrated to pH 7 against a standard alkaline solution. Carbon dioxide is not included in the total acidity.

Method: Potentiometric titration or titration with bromothymol blue as indicator and comparison with an end-point color standard.

Volatile Acidity

Definition: The volatile acidity is derived from the acids of the acetic series present in wine in the free state and combined as salts.

Principle: Carbon dioxide is first removed from the wine. Volatile acids are separated from the wine by steam distillation and titrated using standard sodium hydroxide. The acidity of free and combined sulfur dioxide distilled under these conditions should be subtracted from the acidity of the distillate. The acidity of any sorbic acid, which may have been added to the wine, must also be subtracted.

Fixed Acidity

The fixed acidity is calculated from the difference between total acidity and volatile acidity.

Organic Acids:

Wine organic acids may be separated and simultaneously determined by high performance liquid chromatography (HPLC) or by anion exchange chromatography, followed by spectrophotometric detection.

pH

Principle: The difference in potential between two electrodes immersed in the liquid under test is measured. One of these two electrodes has a potential that is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode. The instrument used is the pH meter.

1.2.5. Acidity Modulation in Wines

The OIV define the limits and the ways to acidify musts and wines (www.oiv.int/en/):

MUSTS and WINES

Definition: increase of the titratable acidity and the actual acidity (decrease of the pH).

Objectives:

a) Production of balanced wines from a sensory point of view

b) To promote good biological characteristics and good keep in quality of the wine.

Prescriptions:

The objectives can be reached:

a) By blending with musts of elevated acidity;

b) With the help of strong cation exchangers in the free form.

c) By the use of chemical procedures

d) By microbiological acidification

e) By electromembrane treatment

CHEMICAL ACIDIFICATION

Definition: Increasing the titration acidity and the actual acidity (decreasing pH) by adding organic acids.

Objectives:

a) Produce balanced wines from the gustatory point of view;

b) Favor a good biological evolution and good maturation of wine;

c) Remedy insufficient natural acidity caused by:

- climatic conditions in the viticulture region, or
- oenological practices which lead to a decrease in natural acidity

Prescriptions:

a) Lactic acids, L(-) or DL malic acid and L(+) tartaric acid are the only acids that can be used;

b) The addition of acids should not be done to conceal fraud;

c) The addition of mineral acids is forbidden;

d) Chemical acidification and chemical de-acidification are mutually exclusive;

e) The acids used must conform to the International Oenological Codex standards

f) Acids can be only be added to musts under condition that the initial acidity content is not raised by more than 54 meq/l (i.e. 4 g/l expressed in tartaric acid). When must and wine are acidified, the net cumulative increase must not exceed 54 meq/l (or 4 g/l expressed in tartaric acid).

1.3. THE ACID TASTE

The acid taste, in both must and wine, is an important feature of flavor (Pilar *et al.,* 2012). The acidity can be either descripted as sourness when sensed. In fact organic acids and pH are the responsible for the sourness and capable of modifying sourness sensation in wines.

The acids are one of the main components of the wine, that gives a fresh taste and help the wine to be aged and preserved. For example the tartaric acid is more present in warmer region while than colder region where malic is predominant (Zeravik *et al.,* 2015). The acidity is usually lower in the white wines from 3.0 to 3.3 pH and higher in red wines 3.3 to 3.6 pH. They have different characteristics and the effect on the palatability is quite different (Jackson, 1994). Depending on the concentration they produce a pleasant and refreshing sensation or unpleasant acidity (Pilar *et al.*, 2012).

During the last century, so many efforts and studies are been done, to understand the chemical base for sour taste and it has been recognized that hydrogen ions dissociated in aqueous solutions, are perceived to be sour (Neta, 2007) (Figure 1.7). However this is not enough for explain the sour taste. Sourness has been shown to vary independently with pH, total acid concentration and specific anion (Sowalsky and Noble, 1998). The sourness intensity of the acids in fact, even not modifying the concentration, increased with decreasing pH, while at different pH level, sourness increased with increasing acid concentration (expressed as normality).

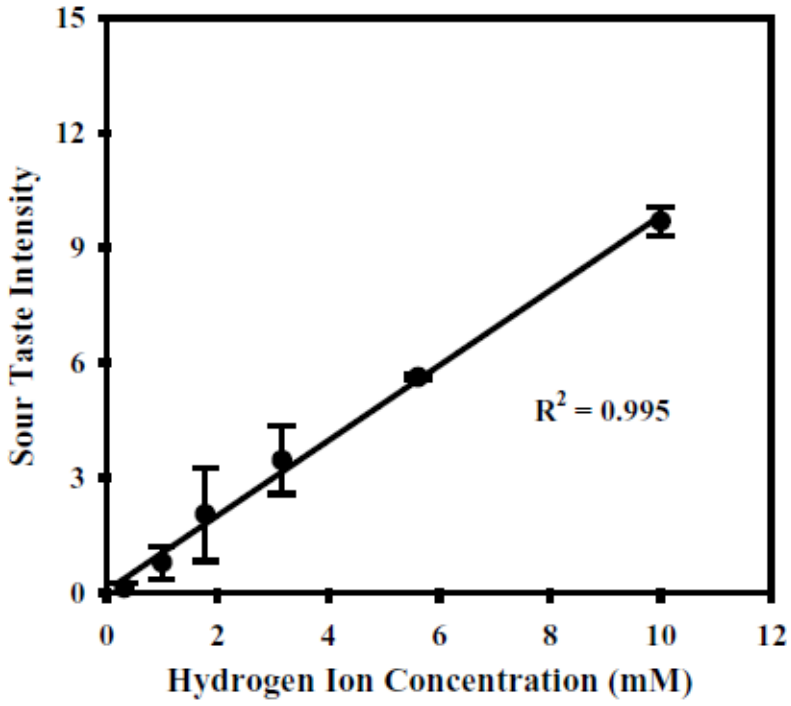


Figure 1.7. Relationship between sour taste intensity and hydrogen ion concentration (Neta, 2007).

Johanningsmeier *et al.* (2005) proposed that the main factor explaining the sour taste intensity of organic acids, is the molar concentration of acid molecules that have one or more protonated carboxyl groups. Neta (2007) instead states that the sour taste of organic acids is directly related to the number of molecules with at least 1 protonated carboxyl group plus the hydrogen ions in solution.

Furthermore protonated organic acid species and hydrogen ions were found to have approximately equal sour taste responses on a molar basis. The acidity, except for the volatile, is possible to perceived it, only by the taste. Speaking about which acidity confer the taste, the literature agrees that what is more important is not the pH, but instead the titratable acidity (Neta, 2007). The problem is that pH and TA are correlated so it's difficult to separate them.

In general the acidity confers freshness, crispy and tart taste. Even if all the main acids in the wines confer the sour taste, they are characterized by different persistence, intensity, aggressivity and saltiness. They even contribute in different path to the fullness of the mouthfeel. The Figure 1.8, adapted from Laffort Tools for Acidification in Musts and Wines, express this concept very well showing the persistence at the abscissa and the intensity at the ordered.

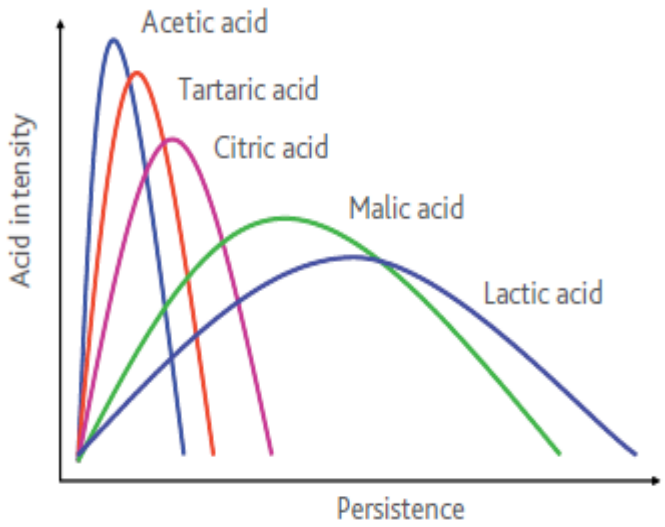


Figure 1.8. Effect of the acids on mouthfeel sensations: Intensity and Persistence. Source: Laffort. Tools for acidification in Musts and Wines.

Interaction of acidity with other compounds

The acidity perception can be influenced by other constituent of the wine such as bitterness, sweetness, and astringency may coexist (Neta, 2007). The presence of ethanol, it has a suppressing effect on sourness. Organic acids (tartaric, lactic, malic, citric and acetic acids) and some inorganic acids can elicit astringent sensations in addition to a sour taste. Non-phenolic organic (e.g. lactic, citric, tartaric, malic, quinic and acetic acids) and some inorganic acids (e.g. hydrochloric and phosphoric acids) can elicit astringent sensations in addition to a sour taste (Bajec and Pickering, 2008).

Sourness and astringency both decrease with the increases of the pH indicating a dependency between those properties (Laaksonen, 2011). In fact the perceived sourness of

various organic acids decreased along with increasing pH (Lugaz *et al*., 2005). The omission of organic acids from a red wine model solution also resulted in a decrease in puckering astringency, but an increase in velvety astringency (Laaksonen, 2011). Sourness and sweetness as well are related. Lowest the quantity of sweet compounds and slightly will increase the acidity.

The pH is able to affect astringency while instead the variation of tartaric acid concentration, at constant pH is not able to effect it. As well the astringency attributed to some acids it was noticed to be related to the pH because neutralizing it with NaOH, the astringency was decreased (Fontoin *et al*., 2008).

1.4. OBJECTIVES OF THE STUDY

As explained before, acidity is one of the most relevant features involved in wine quality and appreciation. Therefore, it is of most importance to evaluate the sensory responses to changes in the acidity of wines, anticipating the next commercial trend of cool climate wines. Although research has already been published in the theme, an approach considering the different taster segments and the different acids is missing.

Therefore, the objectives of this study were:

i) To identify the Detection and Recognition Thresholds for tartaric, malic and lactic acids.

ii) To understand the different levels of appreciation for the different acids.

iii) To assess possible relations between the characterization of the tasters and their responses to wine acidity.

2. MATERIALS AND METHODS

2.1. TASTER SELECTION

The tasting panel was mainly selected among the students of the Master of Viticulture and Oenology of the Instituto Superior de Agronomia (2015/2016). First and second year students were the main targets for the work. The selection has been conducted in order to find the subjects with the best knowledge and sensitivity about the main descriptors of the mouthfeel: acidity, sweetness, bitterness and astringency with the purpose to have a group of people trustable in the results and trained to perceive differences.
The guideline was to find out subjects that consume usually wine at least one for week and able to distinguish the samples submitted. They were prepared, highlighting the main mouthfeel perception. The work started contacting students, males and females, from the ISA without any age limit, smokers and non-smokers.

First Test
To the first call 56 persons came for the selection. The first trial consisted in 9 samples served in 2 groups (Table 2.1).

Table 2.1. Samples used in the first trial: first and second group.

1st Group	2nd Group
1) Sugar (10 g/L) 2) Tartaric acid (1 g/L) 3) Chinine Sulphate (0.0108 g/L) 4) Alluminium Sulphate (0.8 g/L) 5) Ethanol (40%)	6) Lactic Acid (1 g/L) 7) Malic Acid (1 g/L) 8) Aluminium Sulphate (0.8 g/L) 9) Tannins (1 g/L)

1° Group – The compounds were added to distilled water for give the following sensation in order with the table: 1) sweetness, 2) acidity, 3) bitterness, 4) astringency, 5) alcoholic/hot mouthfeel.
2° Group – Served after the first with the purpose of give an idea of the difference between two kind of acidity and two of astringency.
All the solutions where prepare in distilled water, served in white glasses, at room temperature. It was asked to the subjects to describe the sensation felt and write it down.

Second Test

To the second call 56 persons came for the selection. The second trial consisted in 9 samples served in 2 groups (Table 2.2).

Table 2.2. Samples used in the second trial: first and second group.

1° Group	2° Group
1) Tartaric Acid (1 g/L) + Sacarose (10 g/L) 2) Tartaric Acid (1 g/L) + Aluminium Sulfat (0.8 g/L) 3) Tartaric Acid (1 g/L) + Sacarose (10 g/L) + Ethanol (5%) 4) Tartaric Acid (1 g/L) + Sacarose (10 g/L) + Ethanol (5%) + Tannins (1 g/L) (Quertanin Plus)	5) Control (number 4) + Mannoproteins (0.3 g/L) 6) Control (number 4) + Arabic gum(2 g/L) 7) Control (number 4) + CMC (carboximetilcelulosa) (0.2 g/L)

After one week another trial was done. Mixed sensations to evaluate the capacity to identify them, even if not in single presence.

1° Group – In the first group the following sensation were meant to be presented: 1) acidity and sweetness, 2) acidity and astringency, 3) acidity, sweetness and warmness, 4) acidity, sweetness, warmness and astringency.

2° Group – The second group was served using the number 4) as control and as base solution. The purpose was to give an idea of how different can appear a solution treated with different stabilizers.

All the solutions where prepare in distilled water, served in white glasses, at room temperature. It was asked to the subjects to describe the sensation felt and write it down.

Third Test

For the last selection test, it has been used the wines from ISA's winery: white wine (Arinto); red wine (Syrah, Cabernet Sauvignon, Touriga National, Trincadera) (Table 2.3). After have tried the same compounds with distilled water, they were used in white and red wines for compare how the interaction between the used compounds and the ones of the wines, react and give different perception of the same. It has been use the triangular test using white glasses, at room temperature. It was asked to the subjects to describe the sensation felled and write it down.

Table 2.3. Samples used in the third trial.

1° Group
1) White Wine + Sucrose (30 g/L)
2) White Wine + Tartaric Acid (1 g/L)
3) Red Wine + Chinine Sulphate (0.1 g/L)
4) Red Wine + Tannic Acid (1 g/L)

Final Evaluation

The criteria used to evaluate the tasters, has been previously decided and defined. The subjects who answer wrong to two or more feeling recognition, were allowed to try again the day after but allowing no mistakes. Recognizing them all, were able to pass to the next selection test. At the end of all three panel selections, the ones that met these criteria were finally selected and trained at the same time.

Starting from a group of 56 people, we end up selecting 26 of them. Age included between 22 and 46 years old (Average = 25.3; SD = 5.14), 9 females and 17 males, 4 smokers, 6 middle smokers and 16 non-smokers. No one vegetarian and no one with known serious food allergy. In major part were students of the Master in Viticulture and Oenology but even from the bachelor degree and some of the departments of viticulture. All of the subjects were part of the ISA's university, both as students or workers.

2.2. TASTER PHENOTYPING

The PROP (6-n-Propylthiouracil) used in this test was supplied by Sigma. Subjects were asked to evaluate the personal response to the three solutions in order from the low to high concentration: from 0.032 mM to 0.32 mM and then 3.2 mM. 20 ml of each solution was served in standard white glasses at room temperature. The procedure defined to keep the solution in the mouth for a few second, spit it and fill a Magnitude Estimation (ME) line (ISO 11056:1999). Wait for a minute, wash the mouth with water and keep going with the following samples.

The ME line (Figure 2.1) was 102 mm long and the right anchor term was 'strongest imaginable sensation". To evaluate the answers, the length in cm, was calculated from the left edge, to the point marked by the taster in the line.

For comparing trials of acids and wines, the average values were calculated.

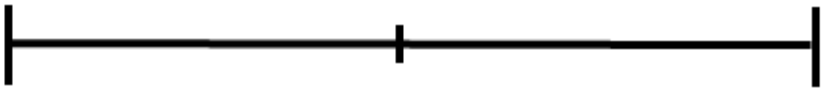


Figure 2.1. ME line (Left border corresponding to the weakest sensation. Middle point corresponding to a medium sensation. Right border corresponding to the strongest sensation. Length 102mm. Middle point at 51mm).

The acids used to add to the wines have different brands. The tartaric acid (L+) was supply by the Panreac AppliChem. The malic acid (DL-) from the Aldrich. The lactic acid (DL-) from Sigma and the citric acid from Merck Millipore.

2.3. VINOTYPE

The subjects were given an online test in order to characterize their relation with the wines and to evaluate their sensitivities and tolerance. 4 different groups were established: sweet, hypersensitive, sensitive and tolerant (www.myvinotype.com).

2.4. SALIVA PRODUCTION

The characterization of the saliva index was applied by administering to the taster 20 ml of a water solution with 4 g/L of citric acid. Subjects were asked to keep in the mouth the whole solution for ten seconds, spit it out, wait another ten seconds and then spit for 60 seconds in a bowl. The final value reported in this work is the average between the two measurements. All the values were used for the comparing in different combinations: using them as normal values and then using a different limit border to create different groups of low-flow rate (LF) and high-flow rate (HF) production saliva tasters. Those borders were use at the levels 2.5, 3 and 3.5 grams (Fischer *et al.,* 1994).

2.5. DETERMINATION OF SENSORY THRESHOLDS

The sensory threshold is “the point at which increasing stimuli trigger the start of an afferent nerve impulse. Absolute threshold is the lowest point at which response to a stimulus can be perceived” (Mosby's Medical Dictionary, 2009). This triangular test is the method for sensory analysis, specified by the International Standards ISO 4120 to detect the differences between samples of two products by triangular comparison. The differences can be all the attributes or just one attribute of the samples.

It can be either used in the selection or training of assessors or either for monitoring the same. Is a very convenient method used in case of low number of assessors available and

there is no risk of sensory fatigue. The principle is based on the simultaneous presentation of a set of three test samples, two of which are identical, for identification of the odd samples. The conditions of the room and the qualifications of the assessors have to respond to the respective ISO 6658.

In case of this test, which it use a significant level of 5%, the number of the assessors can't be lower than 7. The test supervisor can't be part of the test. The samples were served at room temperature.

The subjects were preliminary informed about the test, the working system, the purpose, on which sensory and mouthfeel concentrate with object of avoid any bias in their replies. Casual codes were given in INAO white glass conformed to the ISO. Random distribution of the glasses avoided any possible interpretation of the assessor.

The principle of "forced choice" was applied to choose the sample different from the others products, in order to obtain statistical validity of analysis. The principle of "no difference replies" was not used when it was asked to recognize which the different one was.

BET THRESHOLD

Classically, in sensory research, the best estimate threshold (BET) is used for studying perception value. The Best Estimate Threshold (BET) concentrations for the individual panelists were calculated as the geometric means of the highest undetected concentration and the lowest detectable concentration. The group best estimated threshold was calculated as the geometric mean of the individual thresholds (Panovská *et al*., 2009).

It can happen that, as the panelist became more accustomed to the flavor of the substance and the mechanism of the test, the thresholds could decrease. If this decline is more than 20% we have to repeat the test until the values stabilize (Meilgaard *et al*., 1999).

This method is applied to find out two different thresholds: the Detection and the Recognitions Threshold. The Detection Threshold identifies at which concentration the subject is able to distinguish a sample from the other. The Recognition Threshold identifies at which concentration the subject is able to identify which kind of difference. In the case of the triangular test just one sample is different from the other two. The BET is applied to the answers of every single subjects and then calculated with the final average of the panel. The final result it will be a mean concentration and it will express the value as threshold of distinction. At the same way it is applied for the Recognition Threshold giving a different value concentration. In both cases the BET is the tool through which is possible to calculate and relate the physical intensity of the stimulus to the corresponding sensation and converting to a value (Meilgaard *et al*., 1999).

The BET was used in the trials comparing the different concentration of the same acid and applied to elaborate the results of four different concentration for a single trial. It always been used to work on Arinto wine.

2.6. ACIDS AND ACIDITY APPRECIATION

The purpose of this work was even to find out the appreciation of the tasters in relation with the different acids and acidities. Within the acids, using the Suprathreshold concentration (Duffy and Bartoshuk, 2000) based on the addition of 50% of acids to the Recognition Threshold calculated in the previous test, a comparing of tartaric, malic and lactic acid added was developed. The addiction was made on a base Arinto white wine from ISA's winery. The solutions were served in INAO white glasses at room temperature, 20 ± 2 C°. Four glasses: one white Arinto white wine out of the evaluation to get the mouth used to acidity, and 3 glasses with Arinto white wine and addiction in order of: 1.95 g/L of tartaric acid, 1.5 g/L of malic acid and 1.95 g/L of lactic acid. The ratings were applied through a ME line of 102mm and read as distance from the left border (Annex 10). The parameters evaluated were Intensity, Persistence, Salinity and Appreciation. The average values of every acid were then compared with the others using ANOVA ($P<0.05$) and in case of statistical difference, mean values were compared by Tukey Test, with significant level of 0.05.

For the acidity in wines trial, different samples were used but same methodology. The solutions were served in INAO white glasses at room temperature, 20 ± 2 C°. Four glasses: one white Arinto white wine out of the evaluation to get the mouth used to acidity, the first of the trial with Castelo de Pias (wine from Alentejo), the second with this wine added of 1.5 g/L of malic acid and 1.5 g/L of lactic acid, the third with a Riesling (Germany). The ratings were applied through a ME line of 102mm and read as distance from the left border (Annex 11). The parameters evaluated were Persistence and Appreciation. The average values obtained for every wine were then compared with the others using ANOVA ($P<0.05$) and in case of statistical difference, processed by Tukey Test.

2.7. WINE ANALYSIS

The wines used for the trials has been analyzed in the ISA University Laboratories for the main parameters: density, pH, alcohol, SO_2 free and total, volatile acidity, total acidity, residual sugar and dry extract (Table 2.4). They have been chosen considering the availability of the ISA winery and wanting to use a base wine (Arinto) with a medium acidity level which allows to handle further addictions of acids.

Table 2.4. – Analysis of the wines used in the trials. For concentrations and wines full name see the list below the table.

Wine	*DENSITY*	*pH*	*ALCOHOL STRENGTH*	*FREE SO_2*	*TOTAL SO_2*	*VOLATILE ACIDITY*	*TOTAL ACIDITY*	*RESIDUAL SUGAR*	*DRY EXTRACT*
ARINTO	991.5 g/mL	3.58	11.5 %	18 mg/l	200 mg/l	0.26 g/L	4.2 g/L	1.54 g/L	18.23 g/L
ARINTO +TART	993 g/mL	3.19	11.5 %	18 mg/l	200 mg/l	0.24 g/L	7.2 g/L	1.54 g/L	18.23 g/L
ARINTO +MALIC	993 g/mL	3.29	11.5 %	18 mg/l	200 mg/l	0.27 g/L	7.65 g/L	1.54 g/L	18.23 g/L
ARINTO +LACT	992 g/mL	3.34	11.5 %	18 mg/l	200 mg/l	0.27 g/L	6.15 g/L	1.54 g/L	18.23 g/L
CASTELO PIAS	990.3 g/mL	3.35	12 %	19 mg/l	92,5 mg/l	0.16 g/L	4.5 g/L	1.49 g/L	20.6 g/L
CASTELO PIAS+ACIDS	992.3 g/mL	3.19	12 %	19 mg/l	92,5 mg/l	0.21 g/L	7.05 g/L	1.49 g/L	20.6 g/L
RIESLING	995.6 g/mL	3.04	11.1 %	15 mg/l	65 mg/l	0.1 g/L	9.2 g/L	3.29 g/L	31 g/L

For the panels, seven wines were used. All of them white wines.

- ARINTO, Portugal, ISA'S winery, 2014, 0.4g/L Bentonite.
- ARINTO + TARTARIC ACID: the same Arinto with 3.2 g/L of tartaric acid
- ARINTO + MALIC ACID: the same Arinto with 3.2 g/L of malic acid
- ARINTO + LACTIC ACID: the same Arinto with 3.2 g/L of lactic acid
- CASTELO DE PIAS 2015, Portugal, Amareleza Vinho, LDA 7885-031, 2016
- CASTELO DE PIAS + ACIDS: the same Castelo de Pias with 1.5 g/L of malic acid and 1.5 g/L of lactic acid
- RIESLING, Mosel, Germany, Kabinet, 2015

2.8. STATISTICAL ANALYSIS

To study the results of the trials and their relations with the taster characterization, the analysis of variance (ANOVA) and comparison of treatments means (Tukey's Test) were performed using Microsoft Excel and Statistix 9.0 software, with $\alpha=0.05$. Results were displayed as mean values of the assays. Statistical significance (at $P<0.05$) of the differences between mean values was assessed by Tukey's test.

3. RESULTS AND DISCUSSION

3.1. TASTER CHARACTERIZATION

3.1.1. Taster Phenotype

The evaluation of the taster phenotype was performed using the responses to increasing concentrations of PROP. The results were the average of two measurements and are shown in table 3.1, revealing the expected 3 classes of sensitivity (non-tasters, tasters and super-tasters) as reported by Pickering *et al*. (2004). These authors used the bitterness rating assigned to the 0.32 mM PROP solution as the indicator to distinguish between the 3 classes (non-taster ≤ 15.5; 15.5 < taster <51; super-taster ≥ 51 mm). Accordingly, in our study the individuals were considered mostly tasters (10) and supertasters (13) while there were only 3 non-tasters. The graphical output of the results is a clear indication that our group of tasters showed distinct patterns as a function of PROP sensitivity (Figure 3.1).

Table 3.1. Bitterness ratings of PROP solutions (mM) using the Magnitude Estimation scale.

PROP Status	0.032 mM	0.32 mM	3.2 mM
Non-Taster	4.76 ± 0.45 a	10.54 ± 0.17 c	45.73 ± 3.55 c
Taster	9.28 ± 1.71 a	34.53 ± 3.18 b	77.72 ± 4.46 b
Super-Taster	19.62 ± 4.89 a	72.18 ± 2.97 a	97.72 ± 1.24 a

Values shown are the mean of two determinations ± standard error. For each concentration, means sharing the same indicator (a, b, c) do not differ significantly (p>0.05).

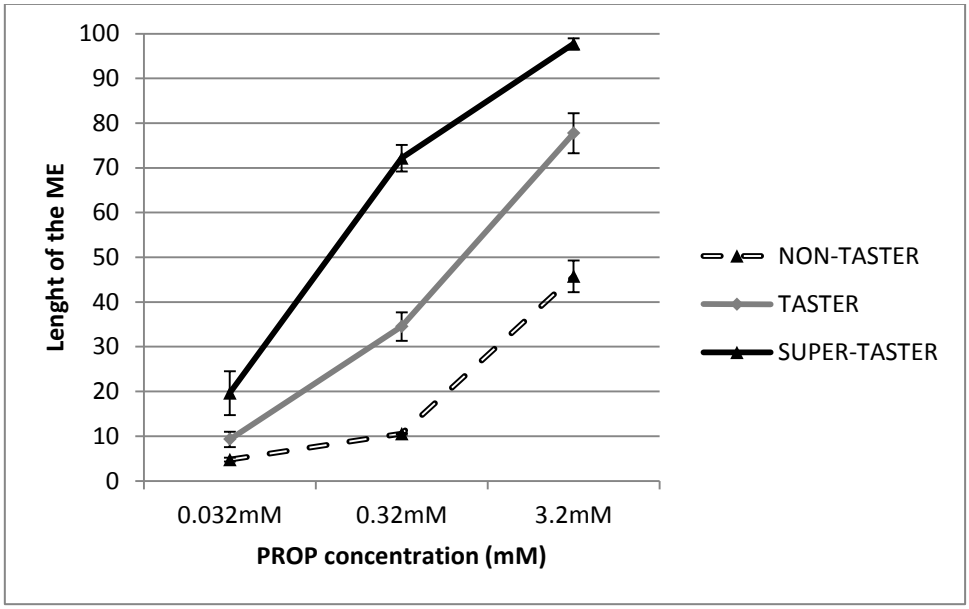


Figure 3.1. Mean bitterness intensity for PROP solutions as a function of PROP concentration, shown separately for non-tasters, tasters, and super-tasters. Error bars indicate standard error.

3.1.2. Vinotype

The responses to the Vinotype questionnaire yielded, on a total of 26 tasters, 0 Sweet, 2 Hypersensitive, 17 Sensitive and 7 Tolerant tasters (Table 3.2).

Table 3.2. Number of tasters according to their Vinotype.

	Sweet	Hypersensitive	Sensitive	Tolerant
Number of Tasters	0	2	17	7

3.1.3. Saliva Production

The amount of saliva produced by the tasters (average of 2 determinations) is shown in Figure 3.2. The lowest value was 1.416 g/min and the highest 4.466 g/min with a total average of 3.2 g/min, with a regular increase in the amount produced within the range.

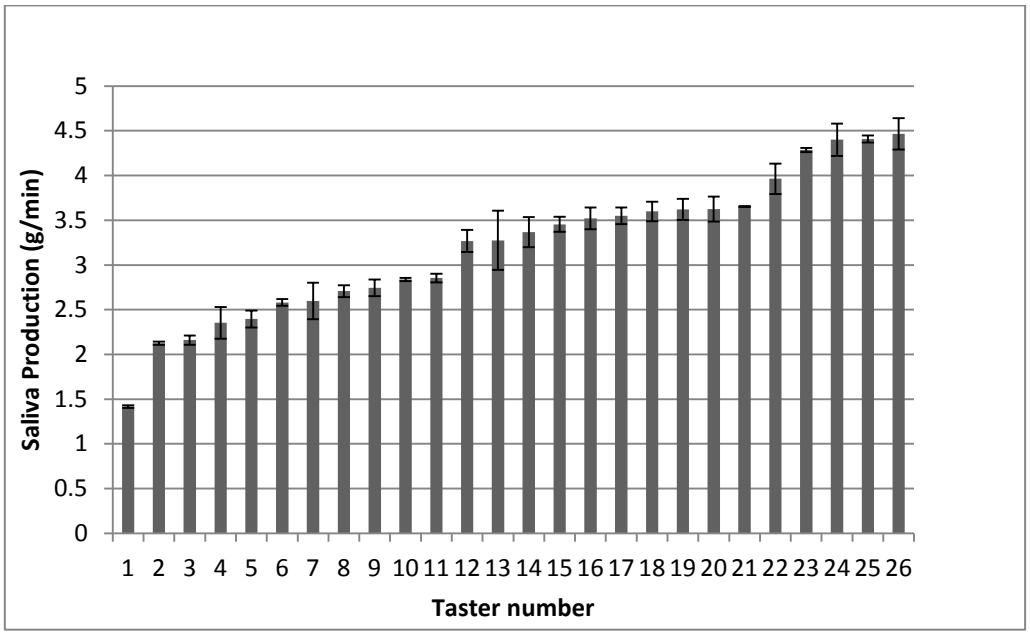


Figure 3.2. Production of saliva (g/min) for each taster. Results are the mean of 2 determinations and error bars indicate standard error (SE).

3.1.4. Overall Taster Characterization

The selected tasting panel was composed by 9 females and 17 males with an average age of 25 years and a standard deviation of 5 years. The overall characterization of the tasting panel using in this work is shown in table 3.3, showing each gender, age, Vinotype, saliva production and PROP sensitivity.

Table 3.3. Demographic and physiological characterization of the tasting panel.

Taster number	Gender	Age	Vinotype	Saliva (g/min)	PROP Sensitivity
1	Female	25	Tolerant	3.521	Super-Taster
2	Male	23	Sensitive	2.744	Taster
3	Female	25	Sensitive	3.963	Super-Taster
4	Male	21	Tolerant	4.408	Super-Taster
5	Female	22	Hipersensitive	2.396	Super-Taster
6	Male	23	Sensitive	4.286	Super-Taster
7	Male	24	Sensitive	3.549	Super-Taster
8	Male	24	Sensitive	3.625	Taster
9	Male	35	Sensitive	2.353	Taster
10	Male	46	Sensitive	2.125	Super-Taster
11	Male	24	Hipersensitive	3.621	Taster
12	Female	22	Sensitive	3.267	Super-Taster
13	Male	24	Sensitive	2.707	Non-Taster
14	Male	24	Tolerant	2.161	Taster
15	Female	22	Sensitive	2.854	Non-Taster
16	Male	32	Tolerant	3.275	Non-Taster
17	Female	28	Tolerant	2.837	Super-Taster
18	Male	22	Sensitive	3.367	Taster
19	Male	24	Tolerant	3.655	Taster
20	Male	26	Sensitive	4.401	Super-Taster
21	Female	22	Tolerant	3.599	Super-Taster
22	Female	23	Sensitive	1.416	Taster
23	Female	23	Sensitive	2.597	Super-Taster
24	Male	23	Sensitive	3.455	Taster
25	Male	26	Sensitive	2.581	Taster
26	Male	26	Sensitive	4.466	Super-Taster

3.2. SENSORY THRESHOLDS OF ORGANIC ACIDS

The determination of taste Detection and Recognition Thresholds was done by the selected tasting panel using four solutions of each tartaric, malic and lactic acid (0.4; 0.8; 1.6; 3.2 g/L) added to an Arinto base wine. The addition of the acids lowered the pH of the solution that was reestablished using the NaOH till the achievement of the original value of 3.58. The sheet used to evaluate the panelist's answers is in annex 9.

3.2.1. Tartaric Acid Thresholds

The concentrations detected as different from the blank or recognized as more acid are shown in table 3.4. These results enabled the calculation of the Best Estimate Threshold (BET) for the detection and for the recognition. The calculated BET for detection was 1.05 g/L while the BET for Recognition was 1.32 g/L (Table 3.4).

Table 3.4. Best estimated threshold (BET) calculation for the Detection and Recognition thresholds of tartaric acid (g/L). Correct choice indicated by 1 and incorrect by 0; highlighted grey cells indicate recognition of acid taste.

Taster	0.4	0.8	1.6	3.2	Detection threshold		Recognition threshold	
1	0	0	1	1	1.13	0.05	1.13	0.05
2	0	0	1	1	1.13	0.05	4.53	0.66
3	0	0	1	0	4.53	0.66	4.53	0.66
4	0	0	1	0	4.53	0.66	4.53	0.66
5	0	1	1	1	0.57	-0.25	4.53	0.66
6	0	1	0	0	4.53	0.66	4.53	0.66
7	0	1	1	1	0.57	-0.25	0.57	-0.25
8	0	1	1	1	0.57	-0.25	0.57	-0.25
9	0	0	1	0	4.53	0.66	4.53	0.66
10	0	0	0	0	4.53	0.66	4.53	0.66
11	0	0	1	1	1.13	0.05	1.13	0.05
12	1	1	1	1	0.20	-0.70	0.20	-0.70
13	0	1	1	0	4.53	0.66	4.53	0.66
14	1	0	0	1	2.26	0.35	4.53	0.66
15	1	1	1	1	0.20	-0.70	0.57	-0.25
16	0	0	1	1	1.13	0.05	4.53	0.66
17	0	1	1	1	0.57	-0.25	0.57	-0.25
18	0	1	1	1	0.57	-0.25	0.57	-0.25
19	1	1	1	1	0.20	-0.70	4.53	0.66
20	0	0	1	1	1.13	0.05	4.53	0.66
21	0	1	1	1	0.57	-0.25	0.57	-0.25
					Log BET	**Antilog**	**Log BET**	**Antilog**
					0.05	1.05 g/L	0.28	1.32 g/L

The detection threshold was also determined graphically as shown in figure 3.3. Considering 12 subjects out of 21 as the minimum number in a triangular test to establish the difference (P=0.05), the interpolated value was 0.91 g/L, similar to the calculated detection threshold.

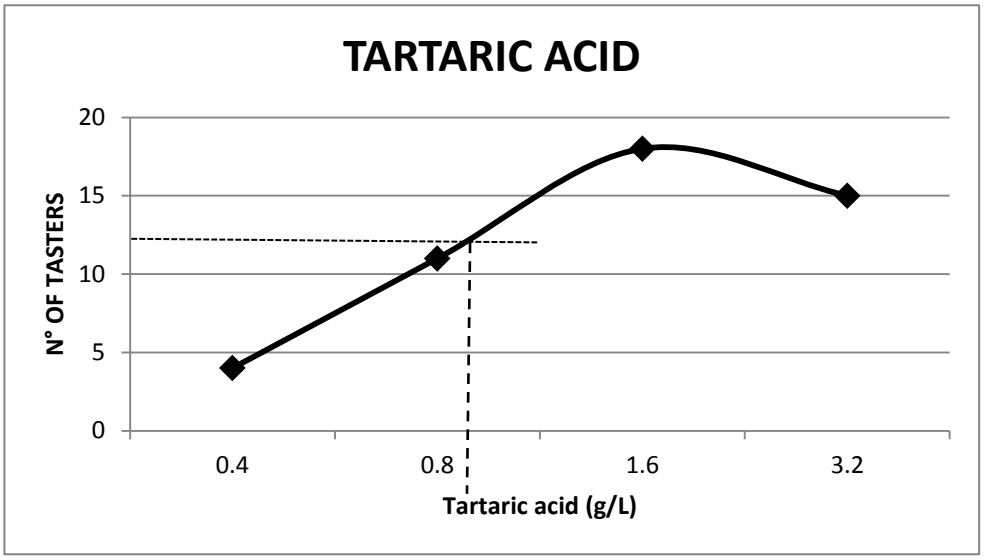


Figure 3.3. Geometric trend of Detection Threshold of Tartaric Acid. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line (n = 12) represents minimum agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (total number of tasters N=21).

3.2.2. Malic Acid Thresholds

The concentrations detected as different from the blank or recognized as more acid are shown in table 3.5. These results enabled the calculation of the Best Estimate Threshold (BET) for the detection and for the recognition. The calculated BET for detection was 0.85 g/L while the BET for Recognition was 1.06 g/L (Table 3.5). The detection threshold was also determined graphically as shown in figure 3.4. Considering 11 subjects out of 19 as the minimum number in a triangular test to establish the difference (P=0.05), the interpolated value was 0.64, similar to the calculated detection threshold.

Table 3.5. Thresholds calculated with BET for Malic Acid

	0.4	0.8	1.6	3.2	Detection threshold		Recognition threshold	
1	1	1	1	1	0.20	-0.70	0.57	-0.25
2	0	0	1	1	1.13	0.05	1.13	0.05
3	0	0	1	1	1.13	0.05	1.13	0.05
4	1	0	1	1	1.13	0.05	4.53	0.66
5	1	1	1	1	0.20	-0.70	0.20	-0.70
6	0	1	1	1	0.57	-0.25	0.57	-0.25
7	1	1	1	1	0.20	-0.70	1.13	0.05
8	0	1	1	0	4.53	0.66	4.53	0.66
9	0	1	1	0	4.53	0.66	4.53	0.66
10	0	1	1	1	0.57	-0.25	0.57	-0.25
11	1	1	1	1	0.20	-0.70	0.57	-0.25
12	0	1	1	1	0.57	-0.25	0.57	-0.25
13	1	1	1	1	0.20	-0.70	0.20	-0.70
14	0	1	1	1	0.57	-0.25	4.53	0.66
15	0	0	1	1	1.13	0.05	1.13	0.05

16	0	1	1	1	0.57	-0.25	0.57	-0.25
17	1	0	0	0	4.53	0.66	4.53	0.66
18	0	0	1	1	1.13	0.05	4.53	0.66
19	1	1	1	1	0.20	-0.70	0.57	-0.25
					Log BET	**Antilog**	**Log BET**	**Antilog**
					-0.17	0.85 g/L	0.05	1.06 g/L

BET, best estimated threshold; correct choice indicated by 1 and incorrect by 0; highlighted grey cells indicate recognition of acid taste.

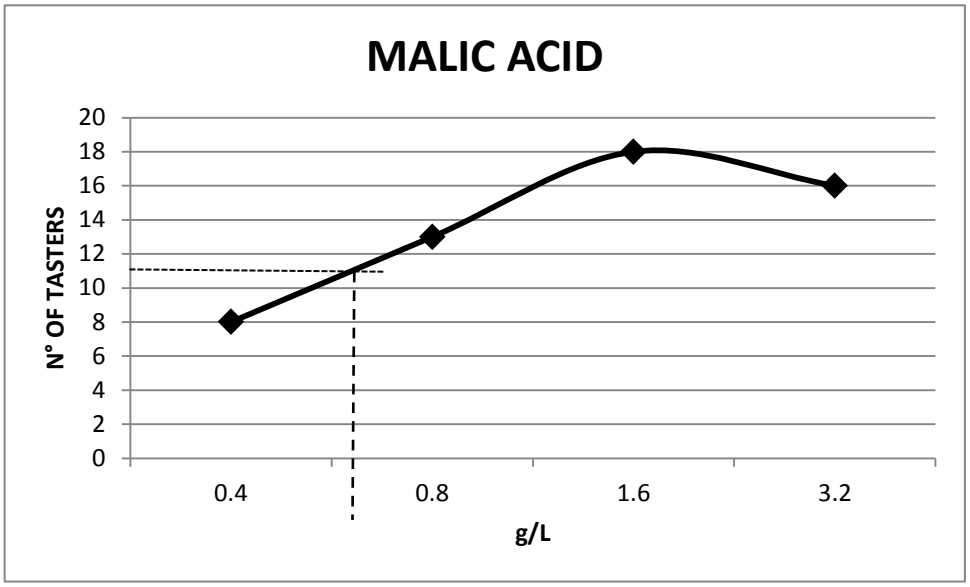


Figure 3.4. Geometric trend of Detection Threshold of Malic Acid. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line (n = 11) represents minimum agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (total number of tasters N=19).

3.2.3. Lactic Acid Thresholds

The concentrations detected as different from the blank or recognized as more acid are shown in table 3.6. These results enabled the calculation of the Best Estimate Threshold (BET) for the detection and for the recognition. The calculated BET for detection was 1.12 g/L while the BET for Recognition was 1.3 g/L (Table 3.6). The detection threshold was also determined graphically as shown in figure 3.5. Considering 10 subjects out of 18 as the minimum number in a triangular test to establish the difference (P=0.05), the interpolated value was 1.23, similar to the calculated detection threshold.

Table 3.6. Trend of Detection Threshold of Lactic Acid.

	0.4	0.8	1.6	3.2	Detection threshold		Recognition threshold	
1	0	1	1	1	0.57	-0.25	2.26	0.35
2	1	0	0	1	2.26	0.35	2.26	0.35
3	0	1	1	0	4.53	0.66	4.53	0.66
4	0	0	1	0	4.53	0.66	4.53	0.66
5	0	0	1	1	1.13	0.05	1.13	0.05
6	0	0	1	1	1.13	0.05	1.13	0.05
7	0	1	0	1	2.26	0.35	2.26	0.35
8	1	1	0	0	4.53	0.66	4.53	0.66
9	1	1	1	1	0.20	-0.70	1.13	0.05
10	0	0	1	1	1.13	0.05	1.13	0.05
11	1	1	0	1	2.26	0.35	2.26	0.35
12	1	0	1	1	1.13	0.05	1.13	0.05
13	1	1	1	1	0.20	-0.70	4.53	0.66
14	0	0	1	1	1.13	0.05	1.13	0.05
15	0	1	1	1	0.57	-0.25	0.57	-0.25
16	0	0	1	0	4.53	0.66	4.53	0.66
17	1	0	0	0	4.53	0.66	4.53	0.66
18	1	1	1	1	0.20	-0.70	0.20	-0.70
					Log BET	**Antilog**	**Log BET**	**Antilog**
					0.11	1.12	0.26	1.30

BET, best estimated threshold; correct choice indicated by 1 and incorrect by 0; highlighted grey cells indicate recognition of acid taste.

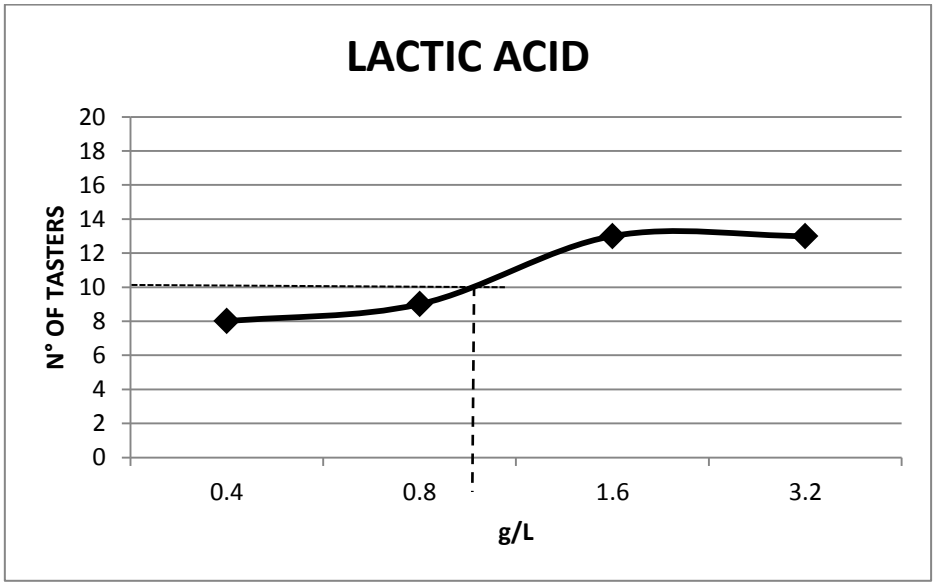


Figure 3.5. Geometric trend of Detection Threshold of Lactic Acid. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line (n = 10) represents minimum agreeing judgements necessary to establish preference using α=0.05 for triangular comparison tests (total number of tasters N=18).

3.2.4. Comparison Among the Thresholds of the Organic Acids

The overall detection responses to the acids are pooled in figure 3.6. As a pattern, tartaric and malic acids induced similar responses while lactic acid seemed to be detected by a lower number of individuals.

The comparison of the thresholds for the acids added to the wine should be done not in mass concentration but in molar concentration. Hence, the Detection and Recognition Thresholds were converted into tartaric acid concentration (table 3.7). The final thresholds were calculated by adding the natural total acidity value expressed as tartaric acid of the base wine. The highest thresholds were determined for tartaric acid, while lactic acid showed the lowest thresholds.

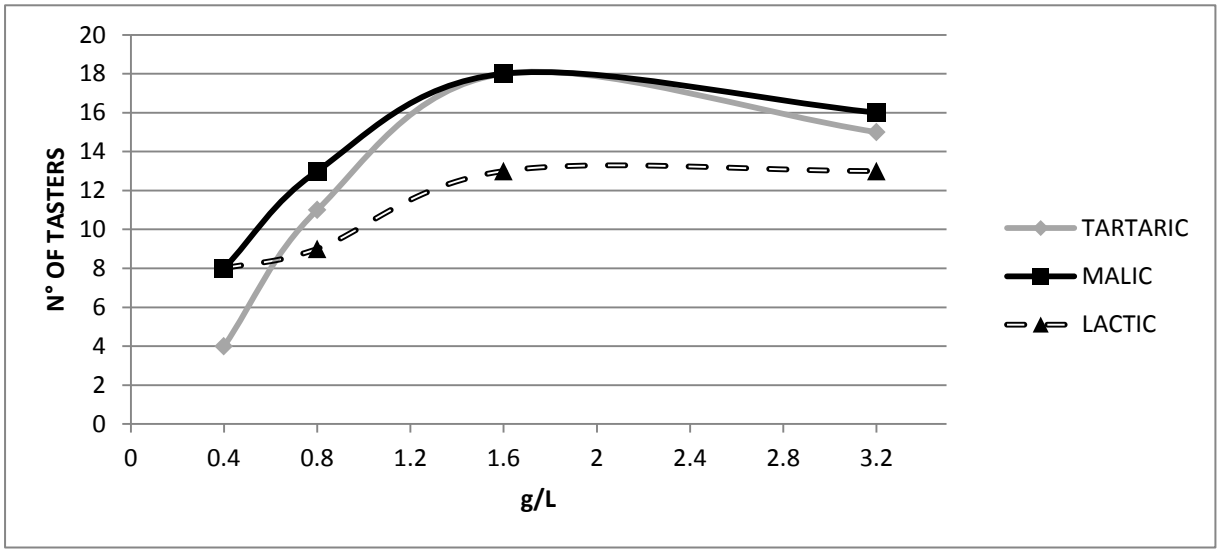


Figure 3.6. Compared logarithmic trends of the three acids. Grey line = Tartaric acid; (♦), black line = Malic acid; (■), dashed line = lactic acid.

Table 3.7. Detection and Recognition Thresholds (g/L) for Tartaric, Malic and Lactic Acid in white wine with 4.2 g/L of total acidity expressed as tartaric acid.

Acid	Detection Threshold	Recognition Threshold	Detection Threshold in wine	Recognition Threshold in wine
Tartaric	1.05	1.32	4.20 + 1.05 = 5.25	4.20 + 1.32 = 5.52
Malic	0.85	1.06	4.20 + 0.95 = 5.15	4.20 + 1.19 = 5.39
Lactic	1.12	1.30	4.20 + 0.93 = 5.13	4.20 + 1.08 = 5.28

3.3. SENSORY RESPONSES TO SUPRA-THRESHOLD ACID CONCENTRATIONS

These trials consisted in a comparison of the sensory responses given to the 3 organic acids under study added to the Arinto base wine. The amount of acids to add was decided using the concept of supra-threshold concentration (Duffy and Bartoshuk, 2000) aiming their easier

perception by all the tasters of the panel. Therefore, we decided to add a concentration 50% higher than the respective recognition threshold. The amounts were: tartaric acid 1.95 g/L, malic acid 1.5 g/L and lactic acid 1.95 g/L.

The tasters were asked to fill a sheet (Annex 10) with a ME scale for each investigated parameter: Intensity, Persistence, Salinity and Appreciation. The evaluation of each taster is shown in Annex 1. Figure 3.7 demonstrates graphically the obtained results and table 3.8 shows the mean scores of each descriptor and their statistical difference. Overall, there were no differences in the intensity, salinity and persistence among the three acids. Concerning the appreciation, lactic acid was preferred in relation to tartaric acid but not in relation to malic acid (ANOVA P=0.0134; Annex 3).

Figure 3.7. Acid Trial Result in mean (Tartaric acid=Arinto plus 1.95g/L of tartaric acid; Malic acid=Arinto plus 1.5g/L of malic acid; Lactic acid=Arinto plus 1.95g/L of lactic acid).

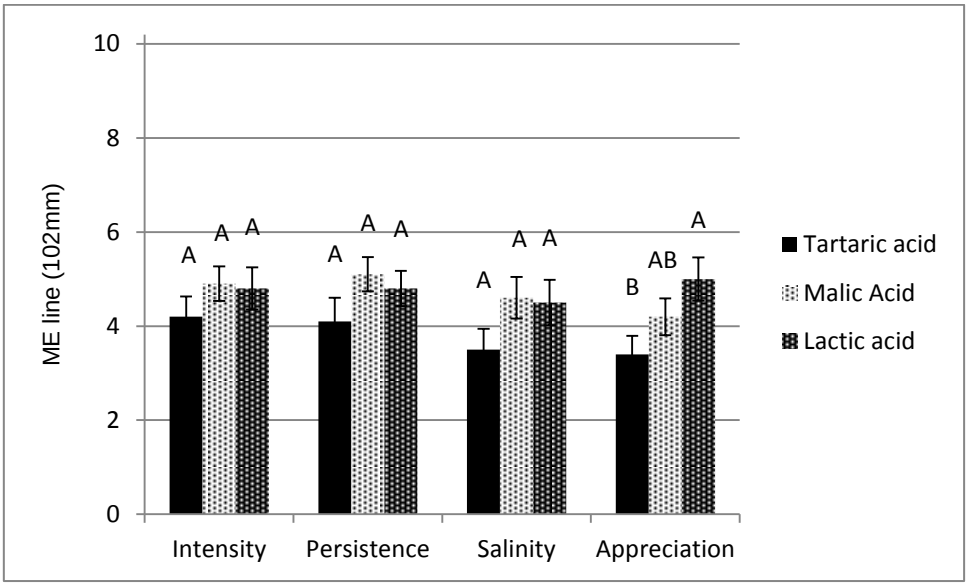


Table 3.8. Acid Trial Result in mean (Tartaric acid=Arinto plus 1.95g/L of tartaric acid; Malic acid=Arinto plus 1.5g/L of malic acid; Lactic acid=Arinto plus 1.95g/L of lactic acid).

Descriptor	Tartaric acid	Malic Acid	Lactic acid
Intensity	4.2 ± 0.43 a	4.9 ± 0.37 a	4.8 ± 0.45 a
Persistence	4.1 ± 0.50 a	5.1 ± 0.36 a	4.8 ± 0.38 a
Salinity	3.5 ± 0.44 a	4.6 ± 0.44 a	4.5 ± 0.48 a
Appreciation	3.4 ± 0.39 b	4.2 ± 0.39 ab	5.0 ± 0.46 a

3.4. SENSORY RESPONSES TO ACIDS ADDED TO DIFFERENT WINES

In this trial we intended to see how the acidification of a low acidity wine from a warm region would compare to a high acidity wine from a cold region. The test has been made with three

different wines: Castelo de Pias (Alentejo, Portugal), the same Castelo de Pias (4.5 g/L total acidity) added with 1.5 g/L of malic acid and 1.5 g/L of lactic acid (7.05 g/L total acidity) and a Riesling (Germany) (9.2 g/L total acidity). The analysis of the wine added with the acids has been reported in table 3.9 included the expected total acidity. The tasters were asked to fill a sheet (Annex 11) with a ME scale for each investigated parameter: Persistence and Appreciation.

Table 3.9. Expected total acidity after acids addition calculated using the multiplying factor.

Wine	Total Acidity	Expected Total Acidity (expressed in Tartaric acid)
Castelo de Pias	4.5	4.5
Castelo de Pias + Acids	7.05	(1.68 + 1.25) + 4.5 = 7.43

They were served to the panel in the order previously mentioned anticipated by a glass of Arinto white wine as a warm-up. The taster responses are illustrated in figure 3.8 and listed in table 3.10, showing that there no recognized differences appreciation of the 3 wines. ANOVA showed only statistical difference regarding Persistence of Castelo de Pias and the Tukey Test confirmed it showing homogeneous groups (Annex 4). The evaluation of each taster is shown in Annex 2.

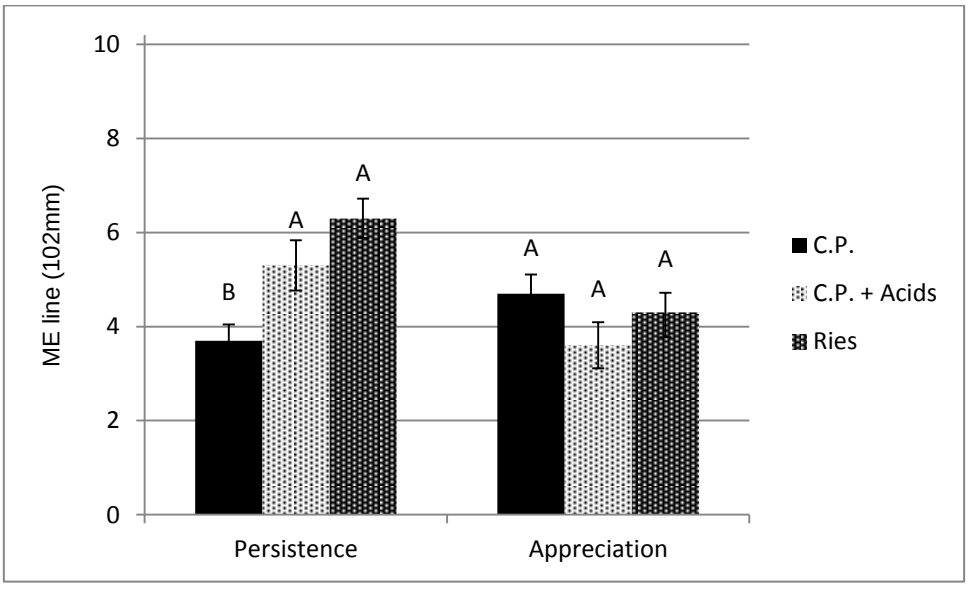


Figure 3.8. Wine trial results (C.P.=Castelo Pias; C.P.+Acids=Castelo Pias plus 1.5g/L malic acids and 1.5 g/L lactic acid; Ries=Riesling).

Table 3.10. Wine Trial Results (C.P.=Castelo Pias; C.P.+Acids=Castelo Pias plus 1.5g/L malic acid and 1.5 g/L lactic acid).

Descriptor	*C.P.*	*C.P. + Acids*	*Riesling*
Persistence	3.7 ± 0.34 b	5.3 ± 0.54 a	6.3 ± 0.42 a
Appreciation	4.7 ± 0.40 a	3.6 ± 0.49 a	4.3 ± 0.53 a

3.5. SENSORY RESPONSES ACCORDING TO TASTER SEGMENTATION

The previous trials yielded mean responses that were not statistically different given the large variation in the responses. We hypothesized that these variability could be reduced if responses were treated according to the different taster segments. Therefore, in order to understand the taster responses we studied the influence of segmentation according to gender, taste phenotype, Vinotype and saliva production.

The analysis of variance (ANOVA) and, within the ones statistically significant, the Tukey test, were used for investigate the possible relations. Positive and negative relations are shown when the variance analysis yields P values lower than 5%. The results are shown in table 3.11.

Table 3.11. Statistical analysis for sensory responses and taster segmentation. N.S.=not significant (P>0.05). S.S.=Statistically significant (P<0.05).

		Vinotype	**Gender**	**PROP**	**Saliva**	**Saliva 2.5**	**Saliva 3**	**Saliva 3.5**
Vinotype		x	x	n.s. 0.9230	n.s. 0.7795	x	x	x
Gender		x	x	n.s. 0.3553	n.s. 0.3342	x	x	x
PROP		n.s. 0.9230	n.s. 0.3553	x	n.s. 0.0957	n.s. 0.6200	n.s. 0.1537	**S.S.** **P=0.0213**
BET	Tartaric	n.s.	n.s.	n.s.	n.s.	**S.S.**	**S.S.**	n.s.,
(Recognition		0.0912	0.9510	0.6467	0.4544	**P=0.0318**	**P=0.0309**	0.3355
Threshold)	Malic	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.0912	0.2126	0.9022	0.2837	0.7437	1.0000	0.3589
	Lactic	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.6488	0.5626	0.8985	0.2377	0.2535	0.4058	0.7304
Acids	Intensity	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.1754	0.5229	0.0925	0.5612	0.9255	0.1391	0.3401
	Persistence	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.8216	0.8264	0.3563	0.5938	0.8308	0.4282	0.3140
	Salinity	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.8909	0.0561	0.3230	0.8753	0.8243	0.3488	0.7299
	Appreciation	n.s.	**S.S.**	n.s.	n.s.	n.s.	n.s.	n.s.
		0.4759	**P=0.0487**	0.2169	0.6348	0.4022	0.6937	0.9199
Wines	Persistence	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.9739	0.7999	0.0880	0.0580	0.2164	0.2465	0.2465
	Appreciation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		0.2465	0.1905	0.0779	0.6303	0.2465	0.5850	0.2164

Relations between the segments

The first ANOVA was made on the segments aiming at finding possible correlations and only the highest saliva producers (>3.5 g) could be related with PROP sensitivity (Table 3.12). The PROP sensitivity (0.32 mM) mean of the highest saliva producers was 62.0 while the lowest producers showed a PROP sensitivity of 39.5 (Annex 5). All the other segments did not show relations among them. In particular we did not find that women, even if most of them are super-taster, were more sensitive to PROP than men as reported by Pickering et al. (2014).

The influence of gender on Vinotype cannot be assessed by variance analysis, as Vinotype is not a quantitative variable.

Table 3.12. Tukey test for relation between Saliva 3.5 (border line between low and high salivators is 3.5) and Prop (0.32mM) show mean value and corresponding class.

SALIVA	PROP (0.32 mM)
Low Producer < 3.5 g/L	39.5 b
High Producer > 3.5 g/L	62.0 a

Relations between the segments and the sensory responses

Gender

The comparison of the gender, showed that there was a relation with the appreciation of the acids distinguishing for the males and females. The males seems to prefer higher concentrations of acid, when the females showed lower liking for high concentrations (Table 3.13). The male showed an average for the appreciation of the acids of 4.5078 (a) and the female 3.4875 (b) and the all 2 means are significantly different from one another (Annex 6). No further relations were found with the others trials and characterizations.

Table 3.13. Tukey test for relation between Gender and Acids Appreciation show mean value and corresponding class.

Gender	Acids Appreciation
Male	4.51 a
Female	3.49 b

Vinotype

Regarding the segments, we could not find any relation between the vinotype and the responses to the wines added of acids. The number of tasters for each classes does not permit to go further with reliable statistical analysis.

Saliva production

The overall saliva production was not related with the sensory responses (Table 3.11) to the acids. However, the division of the tasters according to the level of saliva production yielded different results (Annex 7 and 8). For the variance analysis, we previously segmented the values of saliva in two groups: the ones with total weight below 2.5 g/L and the ones higher than 2.5 g/L (Table 3.14). No statistically significance was evident except for the relation with the result of the Tartaric Acid Bet Recognition Threshold. Those producing more saliva have higher BET. Exactly the same results were highlighted by the comparison between the Tartaric Acid Bet Recognition Threshold and the segmentation of the saliva values, using this time, the 3 g/L as border for the groups (Table 3.14). Those statistically significance is highlighted by the Tukey test that divide the classes. In the table the letters attributed by the Tukey test, divide the classes in “a” as lower saliva producer and “b” as higher saliva producer. This means that the high saliva producers have lower ability to recognize tartaric acid.

Table 3.14. Tukey test for relation between Saliva 2.5 and 3 (border line between low and high salivators is 2.5 and 3) and BET Recognition Threshold Tartaric Acid show mean value and corresponding class.

Saliva production	**BET Recognition Tartaric Acid (g/L)**
< 2.5 g/L	0.95 a
> 2.5 g/L	1.58 b
< 3 g/L	1.70 a
> 3 g/L	3.49 b

3.6. DISCUSSION

Sensory thresholds of organic acids

Our work begun with the establishment of Detection and Recognition Thresholds for the major wine organic acids. Although it seems a very useful tool for their management in the winery the literature is scarce on this subject. According to the recent review of Saenz-Navajas *et al*. (2012), there is no information published on scientific articles regarding the sensory thresholds for tartaric, malic and lactic acids in wines. These authors report their sensory thresholds in water of 44 mg/L, 494 mg/L and 1393 mg/L, respectively, determined using the triangular test. In our study we obtained detection thresholds of about 1 g/L tartaric acid in a wine with 4.2 g/L of total acidity expressed in tartaric acid. Recognition thresholds were about 0.2-0.3 g/L tartaric acid higher, indicating that increases in acidity in concentrations lower than the levels permitted by the OIV, may be easily recognized by the tasters and are effective in changing wine's mouthfeel.

The effect of supra-threshold acid concentrations on wine sensory properties

According to Pickering *et al*., (2004), there is no relation between taster sensitivity, as measured by the previous sensory thresholds, and the response to supra-thresholds concentrations of the tasters. The objective was to find, besides the intensity of the acid taste common to all, further mouthfeel descriptors that could be differently associated with them. Exploratory tastings revealed that persistence and salinity could be more associated with malic and lactic acids, respectively. Therefore, we evaluated the effect of these concentrations on the wine attributes related with acidity (intensity, persistence, salinity) and with hedonic liking (appreciation).

Overall results showed no differences in intensity, persistence and salinity among the 3 acids. However, as a tendency, malic and lactic acids in concentrations 50% higher than their recognition threshold induced higher intensity, persistence and salinity than tartaric acid. Regarding hedonic liking, lactic acid was preferred to tartaric acid while malic acid showed an intermediate appreciation. These results justify the use of malic or lactic acid as acidulants as alternative to tartaric acid in finished wines.

The effect of supra-threshold concentrations of the preferred two acids were then tested in different wines to check if acid addition by itself could turn a low acid wine closer to the characteristics of a naturally sour wine. The addition of malic and lactic acids induced an increase in persistence of the warm climate wine to levels similar to the cool climate wine. However, this was not reflected in the appreciation which was similar for the 3 wines.

The influence of taster segmentation

The gender was shown to influence acid appreciation. Males (17 subjects) preferred higher acidity when compared with females (9 subjects). This is an important answer indicating that males are more inclined to appreciate higher amount of acids when instead the females prefer less sour wines. As far as we are aware this is the first report showing gender segmentation for sourness.

The Vinotype characterization could not be related to acid appreciation. This self-reported questionnaire has been not tested under controlled conditions. Results from our group (Sena-Esteves *et al*., 2016) showed that Sweet and Hipersensitive tasters preferred sweetened wines with 32 g/L sugar, compared to Sensitive and Tolerant tasters. As we had most of the tasters in these two groups, further tests should be done with a wider number of Sweet and Hipersensitive tasters.

The evaluation of the PROP phenotype has been related to genetic sensitivity to sugar and fat (Drewnowski, 1998), to astringency perception (Pickering et al., 2004), to hedonic response to sweet (Drewnowski, 1997). We have only got 3 non-tasters among the enology students which may be explained by the apparent self-selection of tasters and super-tasters among wine experts (Hayes and Pickering, 2012). It seems that there is an active gene-environment correlation explaining why wine experts rate PROP as more bitter than novices (Pickering et al., 2013).

We have found few references to the relation between PROP sensitivity and sensory responses to acidity. In 3 different red wines, Pickering et al. (2004) found out that tasters and super-tasters gave higher intensity ratings than non-tasters for three attributes (bitterness, acidity and astringency). In this work, the intensity ratings of the three acids didn't show any statistical differences (P=0.0925) even if the ANOVA analysis indicated similar mean values for taster (4.85) and super-taster (4.91), but different for the non-tasters (3.27). However, we found that, considering always the mean values, tasters (4.69) had higher levels of appreciation while non-tasters (3.61) and super-tasters (3.9) behaved similarly. In our case the wine was the same, while with Pickering *et al.* (2004) the different red wines varied not only in acidity but also on bitterness and astringency which may have blurred the sole influence of acidity.

Rinaldi *et al.* (2012) and Smith *et al*. (1996) related the SPI (Saliva Precipitation Index) and the amount of saliva, respectively, with the astringency perception of red wines but we did not find reports on the acidity taste. The segmentation according to the whole range saliva production characterization could not be related to sensitivity or appreciation of sourness. However, separating the tasters in several ranges of saliva production showed that the

higher producers (>2.5 and >3.0 g/min) had higher sensitivity to tartaric acid. This could mean that probably the sensibility to the acids, and in particular in this case with the tartaric acid, is related with the quantity of saliva. According to PROP segmentation, high saliva producers (>3.5 g/min) had high sensitivity to bitterness. Although lacking more detailed investigation, this could mean that sensitivity to acid is higher in individuals with high sensitivity to bitterness associated with high saliva production.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

In this work we evaluated the sensory responses given by a trained panel to changes in wine acidity. The determination of detection and recognition thresholds in wine showed that the main organic acids (tartaric, malic and lactic acids) had similar values. The values of both thresholds (~1 to ~1.3 g/l expressed as tartaric acid) demonstrate that relatively small additions may be detected in white wines. The effect of supra-threshold concentrations on wine acidity, persistence and salinity was similar for the 3 acids. However, the higher score for appreciation was given to lactic acid. When wines with different natural acidity were tasted, increasing acidity in “flat” wines do not necessarily increases their preference. Further tests should be done to understand the reactions to increasing acidity in order to help winemakers in their decisions.

The segmentation of the tasters has indicated that uncommon patterns were highlighted. Their characterization, intended to foresee relations between the taster subjectivity and the responses to acidity, gave not positive answers except for some of them. The relation among Gender and Acids Appreciation, where male showed to prefer higher levels of acidity instead of the females, suggests a first parameter of discrimination to bond acidity and subjects features. The relation among the amount of produced saliva and the sensibility to the tartaric acid suggest that the tasters with higher production are more sensitive to the effect of the tartaric acid, considering it having the higher MW. It could suggest that its intensity and aggressiveness it is more perceived from the subjects with higher saliva production. The relation among the saliva 3.5 production and the PROP sensibility, indicates that higher saliva producer are more sensitivity to the PROP bitterness. This is showed even when reading the trend of the other two saliva groups (2.5 and 3) where the P calculated from the ANOVA goes decreasing. All the others segmentation indicates no further statistical significance, giving no clues to predict the responses to the different acidities and their amounts.

Thesis results could be due to the use of trained students, that may have limited the range of taste sensitivities and preferences. A future survey, including also a range of consumers, could indicate the way barely glimpsed in this work.

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ANNEX

	TART	MAL	LAC	TART	MAL	LAC	TART	MAL	LAC	TART	MAL	LAC
	INTENSITY			PERSISTENCE			SALINITY			APPRECIATION		
1	2,8	6	1,4	6,3	3,5	2,8	1,4	6,1	4,7	5,5	1	3,6
2	3,1	5,2	6,6	5,6	6,4	5,1	1,5	2,5	4,1	4,7	6,4	5,4
3	2,5	4,9	8,5	4,5	3,1	5,6	3,6	5,8	4,5	6,7	4,5	2,1
4	8,2	6,3	4,5	8	5,7	4,4	1,1	8,4	2,2	2,7	5,9	9
5	2,2	5,1	5,9	4	6,8	5,1	1,5	3,3	2,4	4	6,2	5,7
6	5,1	4	5,9	0,4	5,2	4,1	3,5	1,3	0,6	2,3	1,5	5,3
7	8,2	10,2	3,9	6,4	9,4	5,1	8,7	3,2	2,1	0,4	0,7	3,5
8	6,6	5,9	5,2	5,9	4,7	6,8	5,2	4,7	6,8	7	5,9	7,6
9	3	6,3	3,9	2,1	6	1,1	4,7	2,8	1,6	1,8	3,3	6,8
10	0,8	4,7	2,5	2	4,7	7,5	2,1	7,5	4,7	2,7	5,4	8,8
11	7,8	5,5	4,9	9,2	3,3	4,3	0,7	8	6,5	1,2	3,2	7,4
12	2,6	1,7	1,3	1,3	0,9	4,6	0,4	0,8	1,6	3,8	2	4,1
13	0,8	3,4	5,5	1,4	4,3	6,3	3,6	4,4	1	3,8	6	1,8
14	2,1	3,7	6,8	3,4	4,3	5,3	3,8	6,4	8,3	3	2	4,3
15	1,3	6,1	3,8	2,2	8,7	5,3	3,5	1,2	5,9	4,4	1,2	7,7
16	6,2	4	1,4	6,3	2	3,8	5,6	1,4	2,4	3,4	1,3	2,1
17	5,1	6	6,8	2	4	6,7	4	5,6	7,5	4,1	6,1	2,7
18	3,8	4,8	6,1	7,7	6,1	6,8	4,8	6	7,6	7,8	4,8	6,6
19	6,2	2,7	1,2	1,3	5,9	4,7	5,6	2,6	6,5	0,9	4	2,4
20	4,2	6	7,5	2,3	4,8	5,8	3,7	7,6	7,3	2,7	4,4	1,8
21	3,6	5,6	7,1	3,6	6	7,1	4,4	5,9	7	1	4,3	6,5
22	4,2	2,6	0,9	4,4	3,1	0,9	5,7	2,2	3,9	4	6,7	1,4
23	5,9	1,3	8,3	9,1	5,9	3,1	8,8	4,4	0,6	0,3	7	4,4
24	5,8	3	4,7	2,7	6	0,3	1,2	4,7	7,5	2,6	4,7	7,5
25	2,6	4,3	3,5	2,1	6,1	4,1	1,2	6,1	4,7	1,8	6	4,1
26	3,6	7,3	6,4	3	5,2	7,1	1,6	5,8	4,4	5,1	3,8	7,1

Annex 1 – Acid Trial Results

	C.P.	C.P. + AC	RIES	C.P.	C.P. + AC	RIES
	PERSISTENCE			APPRECIATION		
1	6,1	3,2	5	4,7	1,3	3,1
2	5,3	6,3	3,7	3,9	3,1	8,1
3	4,9	2,5	5,7	4,4	2,6	1,7
4	3,6	6,3	8,5	3,3	6,1	8,1
5	1,9	6,6	5	2,2	5	3,5
6	1,5	7,2	8,5	5,8	4	7,2
7	2,2	9,6	7,5	6,6	2,9	0,7
8	2,8	6,1	8,4	6,2	9,2	4,1
9	1,7	3,1	3,8	5	3,3	2,5
10	4,9	2,3	7,3	4,9	2,4	6,8
11	0,7	9	7,9	8,1	0,3	4,1
12	4,2	2,5	3,6	1,5	4,7	3,2
13	3	2,1	5,4	1,5	5,6	6,3
14	3,3	4,1	4,9	4,1	1,6	1,1
15	7,5	0,6	2,5	9,1	0,5	1,6
16	3,4	5,7	7,1	2,4	3,7	4,9
17	2,7	7,2	4	6,3	1,9	5,6
18	2,5	7,8	6,6	2,3	7,7	6
19	4,9	8,7	9,6	3,5	0,9	0,3
20	4,7	7,8	6,2	4,1	5,1	1,3
21	2	5	8,8	5	7,3	1,3
22	5,6	1	9,4	7,4	0,7	8,4
23	1,8	2,4	3,3	4,9	1,6	8,6
24	4,7	6,5	7,8	3,7	6,8	5,7
25	5,6	8,2	6,6	7,1	2,8	3,5

Annex 2 – Wine Trial Results

ANNEX 3 – Statistical analysis of acids

	ANOVA					*TUKEY*			
	P(0.05)	*MEAN*			*SE*	*MEAN*			*SE*
		TART	*MALI*	*LACT*		*TART*	*MAL*	*LACT*	
INTEN	0.3132	4.164	4.96	4.944	0.4189				
PERSI	0.1945	4.112	5.16	4.916	0.4237				
SALIN	0.1461	3.448	4.66	4.5	0.4685				
APPRE	**0.0134**	3.348	4.064	5.132	0.4195	3.348 B	4.064 AB	5.132 A	0.5933

ANNEX 4 – Statistical analysis of wines

	ANOVA					*TUKEY*			
	P(0.05)	*MEAN*			*SE*	*MEAN*			*SE*
		CP	*CP+A*	*RIES*		*CP*	*CP+A*	*RIES*	
PERSI	**0.003**	3.66	5.272	6.284	0.4392	**B**	**A**	**A**	0.6212
APPRE	0.2804	4.72	3.644	4.308	0.4772				

ANNEX 5 – Relation between Saliva 3.5 and PROP 0.32mM

	ANOVA				*TUKEY*	
	P (0.05)		*MEAN*	*SE*		*SE*
SALIVA 3	**0.0213**	*SALIVA<3*	39.525	6.0404	**A**	9.1063
		SALIVA>3	62.035	6.8145	**B**	

ANNEX 6 –Gender with Acids Appreciation

	ANOVA				*TUKEY*	
	P (0.05)		*MEAN*	*SE*		*SE*
GENDER	**0.0487**	*MALE*	4.5078	0.2878	**A**	0.5078
		FEMALE	3.4875	0.4195	**B**	

ANNEX 7 – Relation between Saliva 2.5 and BET Recognition Threshold Tartaric Acid

	ANOVA				*TUKEY*	
	P (0.05)		*MEAN*	*SE*		*SE*
SALIVA 2.5	**0.0318**	*SALIVA<2.5*	0.9532	0.2336	**A**	0.2698
		SALIVA>2.5	1.5811	0.1349	**B**	

ANNEX 8 – Relation between Saliva 3.0 and BET Recognition Threshold Tartaric Acid

	ANOVA				*TUKEY*	
	P (0.05)		*MEAN*	*SE*		*SE*
SALIVA 3	**0.0309**	*SALIVA<3*	1.1510	0.165	**A**	0.2333
		SALIVA>3	1.6973		**B**	

ANNEX 9 – Sheet for the thresholds determination

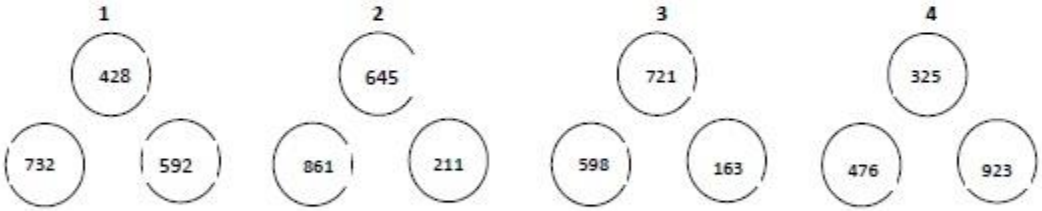
WINE TASTING – PANEL TEST

THRESHOLD DETERMINATION

Name:_____ Surname_____

Age_____ Vinotype_____

1.1) Taste each set and, inside each one, tell which is the different glass and why. If you are not able to identify the different one, don't leave it white but choose one anyway. After you have finished with one set and move to the next, you cannot come back to the previous ones.



1.2)

	N° of the different one	Reason why is different
1		
2		
3		
4		

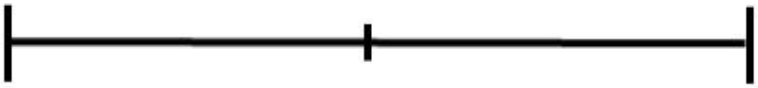
ANNEX 10 – Sheet for the acids comparison

Name _____ Surname _____

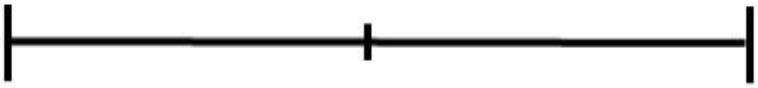
Date_____

WINE PREFERENCE AMONG DIFFERENT ACIDITY

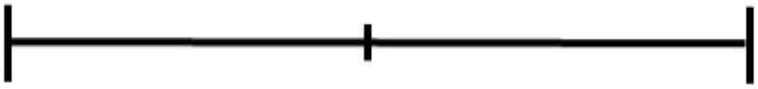
INTENSITY



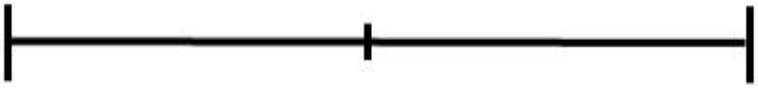
PERSISTENCE



SALINITY



APPRECIATION

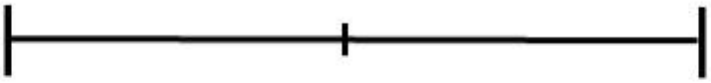


ANNEX 11 – Sheet for the wines comparison

Name _____ Surname _____

Date_____

COMPARISON BETWEEN DIFFERENT WINES, FROM DIFFERENT REGIONS AND DIFFERENT CLIMATES

PERSISTENCE 

PREFERENCE